博士論文

Problems in Trace Analysis of Pesticide Using GC-MS, and Approach towards their Improvements

GC-MS の高感度化に伴う農薬分析上の問題点
及び改善へのアプローチ

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Contents

List of Abbreviations

Chemicals

PEG 300	polyethylene glycol 300
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
TMCS	trimethylchlorosilane
FAME	fatty acid methyl ester

Methods and Instruments

GC	gas chromatograph (instrument)
	gas chromatography (analysis)
GC-MS	gas chromatograph-mass spectrometer (instrument)
	gas chromatography-mass spectrometry (analysis)
GC-MS/MS	gas chromatograph-tandem mass spectrometer (instrument)
	gas chromatography-tandem mass spectrometry (analysis)
LC-MS	liquid chromatograph-mass spectrometer (instrument)
	liquid chromatography-mass spectrometry (analysis)
LC-MS/MS	liquid chromatograph-tandem mass spectrometer (instrument)
	liquid chromatography-tandem mass spectrometry (analysis)
QuEChERS	Quick Easy Cheap Effective Rugged Safe
SPE	Solid Phase Extraction
GPC	gel permeation chromatography
C18	octadecyl
NH_2	aminopropylsilanized silica gel
PSA	ethylenediamine-*N*-propylsilanized silica gel

GCB	graphite carbon black

Others

m/z	mass to charge ratio
MRL	Maximum Residue Levels
LOD	limit of detection
LOQ	limit of qualification
Log $P_{o/w}$	*n*-octanol/water partition coefficient
AMDIS	Automated Mass spectral Deconvolution and Identification System
NIST	National Institute of Standards and Technology
RI	Retention Index
RT	Retention Time
RTL	Retention Time Locking
PLS	Positive List System
MHLW	Ministry of Health, Labour and Welfare
FDA	U.S. Food and Drug Administration
CFDA	California Department of Food and Agriculture

General Introduction

Due to the high sensitivity of a gas chromatograph-mass spectrometer (GC-MS), the trace level analysis of pesticides using GC-MS has become common in the environmental and food safety fields. Because the interests in environmental risk and food safety are growing, the requirements of the number of measured compounds and their detection limits have become very strict. Recently, a gas chromatograph-tandem mass spectrometer (GC-MS/MS) has been widely used as a more selective instrument to meet these strict requirements. The multi-residue analysis of pesticides with wide physicochemical properties has also been common along with the popularization of GC-MS. The molecular weight and Log $P_{o/w}$ value of the pesticides, which are measured by GC-MS, are in the range of 90-541 and -1.2-9.6, respectively.

The water supply regulation by the Ministry of Health, Labour and Welfare (MHLW) was amended in 2004,[1)] the measured pesticides increased from 13 to 102 (Fig. 1), and five metabolites of fenthion were added in 2006. The Positive List System for food safety by the MHLW was in force in 2006,[2)] and the number of measured pesticides has been dramatically increasing (Fig. 2). Before 2005, 250 pesticides were regulated based on the Maximum Residue Levels (MRLs) under the Food Sanitation Law. Under the Positive List System (PLS), the measured pesticides are almost all that are used around the world (around 800 pesticides). The uniform limit (0.01 ppm) has been established for pesticides without MRLs. Approximately 65-70% of the pesticides are measured by GC-MS for both the drinking water regulation and the Positive List System for foods.

It is often said that GC-MS is a matured instrument. However, there are still some problems which influence the quantitative values for trace level analysis of pesticides.

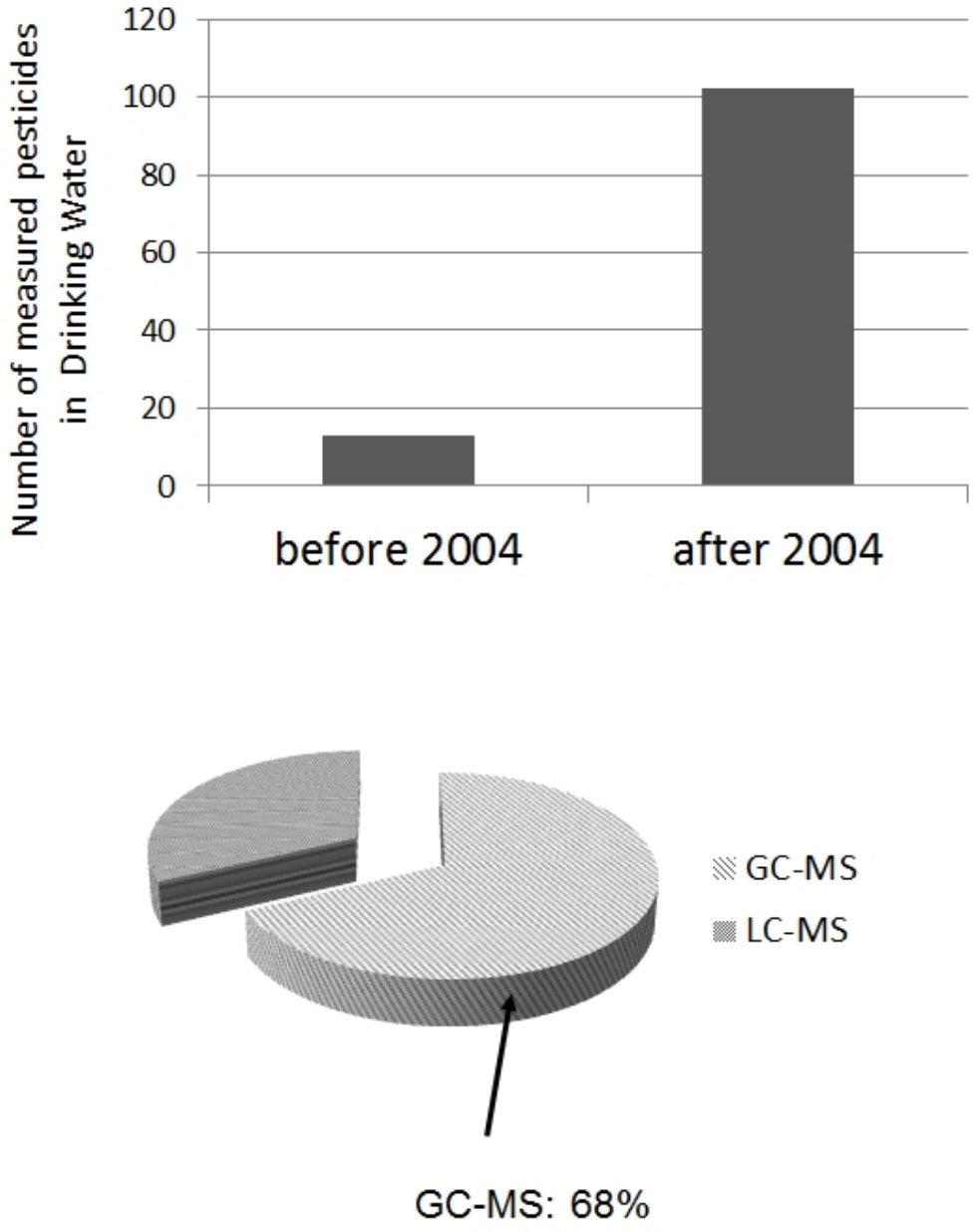


Fig. 1 Increase the number of measured pesticides in drinking water, and the ratio of GC-MS and LC-MS.

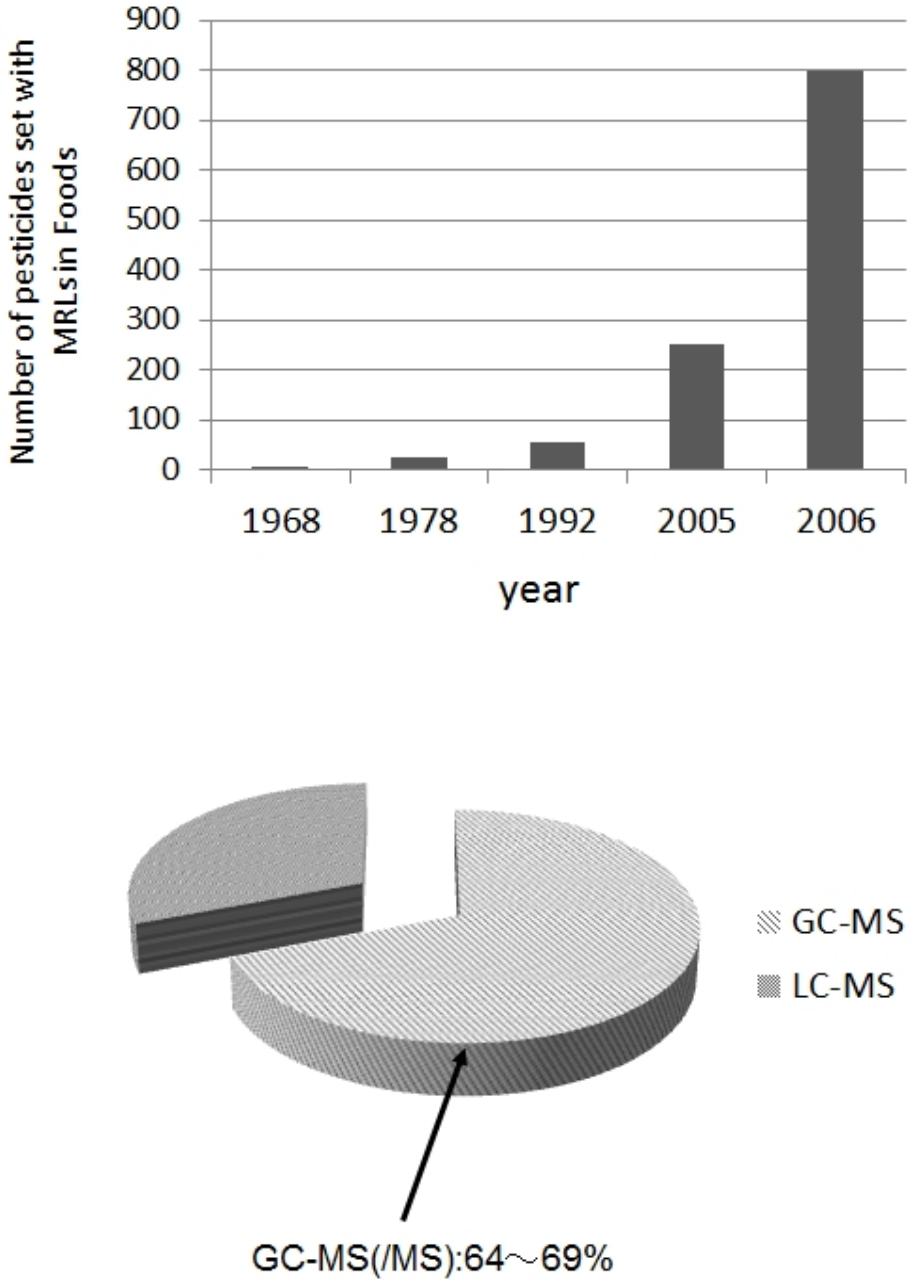


Fig. 2 Increase the number of pesticides set with MRMs in foods, and the ratio of GC-MS and LC-MS.

One of the most serious problems is the matrix enhancement effect such that the intensity of the pesticides in the matrix solution is higher than that in the matrix-free solution.[3)-13)] The matrix enhancement effect is suspected to occur at all the places that are in contact with the pesticides solution; such as the glass vial, injection port, column and ion source. (Fig. 3) The other problem is the change in the mass spectra of specific pesticides, i.e., sulfoxides. Sulfides and sulfoxides have been analyzed by oxidization to sulfone by potassium permanganate treatment[14)] because both are easy to be oxidized. However, since the toxicities of sulfide, sulfoxide and sulfone are different, separate measurements are currently required.

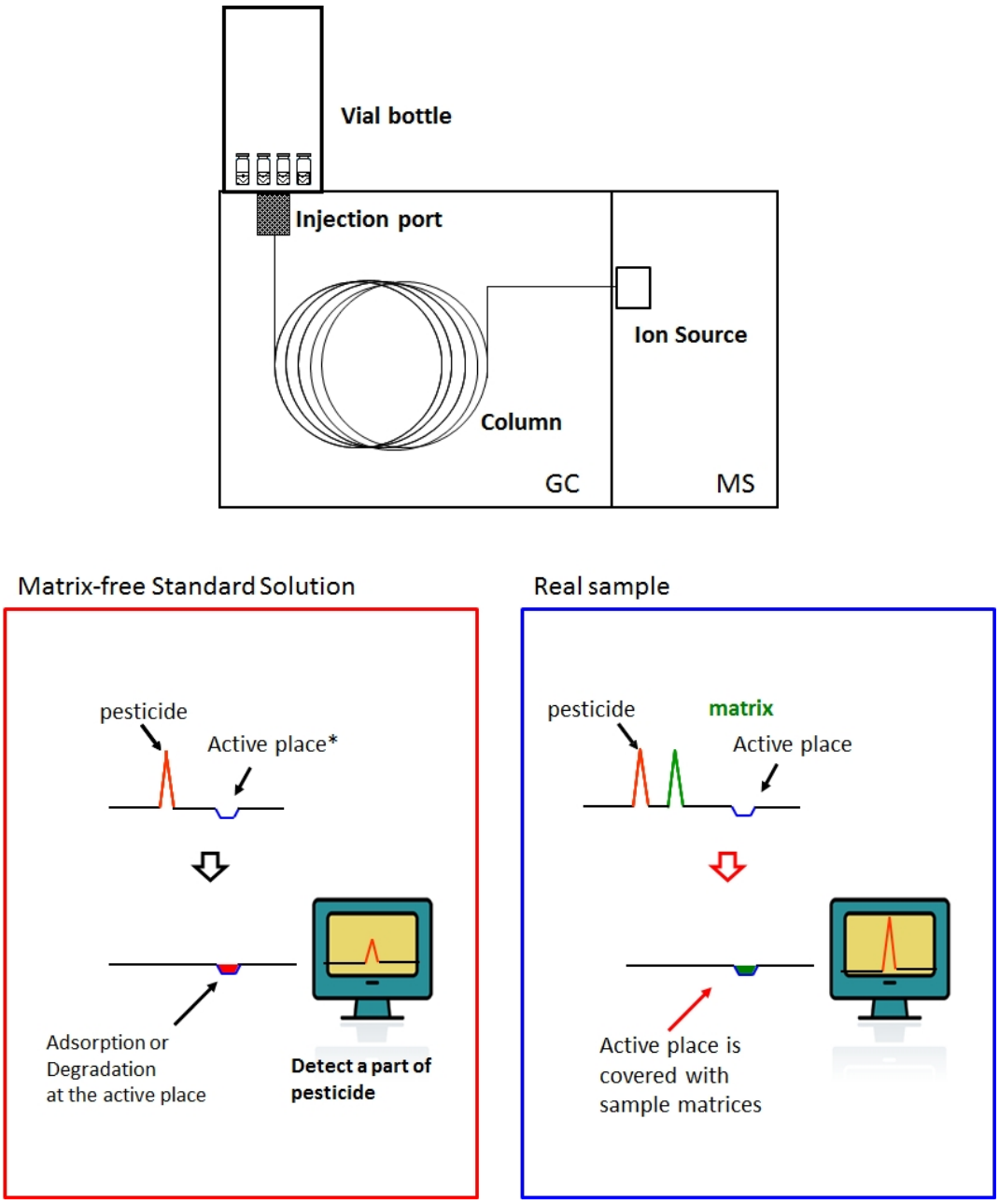
Therefore, the purpose of this thesis is to reveal the cause of these problems and to find a good solution to improve them, from both the aspect of hardware and sample preparation.

In Chapter 1, the change in the mass spectra of fenthion sulfoxides and fensulfothion is described. Two metabolites of fenthion, fenthion sulfoxide and fenthion oxon sulfoxide, are regulated by the drinking water law. Fensulfothion, another pesticide, also has the sulfoxide structure. These sulfoxides have different base peaks depending on their concentrations. The change in the mass spectra is a serious problem for quantitative analysis, and the author determined their mechanism and the solution.

Chapters 2 and 3 describe the matrix enhancement effect problem. A gold-plated ion source was made and the aspect of the hardware was evaluated. We can commercially select a more inert injection liner and column, however, there is no choice about the ion source. That was described in Chapter 2. In Chapter 3, the component which causes the matrix enhancement effect is described using different types of foods.

In Chapter 4, examining the matrix behavior during sample preparation is described. Knowing how the matrix components behave in each step is important to order to a way

to solve the matrix enhancement effect.



*Active place = residual silanol group or places which are not deactivated completely

Fig. 3 Suspicious place where the matrix enhancement occurs and the mechanism.

Chapter 1

Deoxidation of Fenthion Sulfoxide, Fenthion Oxon Sulfoxide and Fensulfothion in Gas Chromatograph/Mass Spectrometer, and the Prevention of Sulfoxide Deoxidation by Polyethylene Glycol 300

1.1 Introduction

Fenthion is one of the organophosphourus pesticides, and is widely used as an effective insecticide for rice, fruits, etc. On the other hand, fenthion raises serious concern because of its strong toxicity to wild birds.[15] Fenthion is photooxidized to fenthion sulfoxide in the environment,[16] and fenthion sulfoxide shows higher toxicity than fenthion.[17]

In Japan, five derivatives of fenthion (fenthion sulfoxide, fenthion sulfone, fenthion oxon, fenthion oxon sulfoxide and fenthion oxon sulfone) are controlled by the water supply law.[1] We had several chances to determine fenthion and its five derivatives, and noted that two sulfoxides sometimes showed mass spectra different from those in the NIST Mass Library (National Institute of Standards and Technology, USA), that is, each base peak shifted to a lower level by 1 *m/z*. We also found that fensulfothion, which is not a fenthion derivative, but has a sulfoxide structure, also acted in the same manner. We extensively examined the data on those compounds to find similar analytical situations.

The base peak of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion in the NIST library, are *m/z* 279, 263 and 293, respectively. In contrast, in the acquired mass spectra, the base peak of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion were *m/z* 278, 262 and 292, respectively. The base peaks shown in the NIST library are derivered from demethylation, which is very popular for EI fragmentation. On the

contrary, the 1 *m/z* lower base peaks are slightly unique because the difference based on molecular weight (mono-isotopic) is an even number for all sulfoxides. In this report, we identify the base peak equal to that of the NIST library as the "usual base peak", and call the 1 *m/z* lower base peak the "shifted base peak". Properly choosing the quantification ion is essential for precise GC-MS quantitative analyses; thus, it is very important to comprehend the cause of producing the shifted base peak result.

GC-MS analyses of sulfoxides were difficult for the following reasons: 1) Sulfoxide is easily produced from sulfide by oxidation, and tends to change into sulfone by additional oxidation. 2) Sulfoxide has a higher polarity than sulfide or sulfone because of its strong polarization between sulfur and oxygen. Fedrak *et al.*[18] reported that methylbenzothiophene sulfoxides were decomposed in a GC injection port. Tanaka *et al.*[19] reported that disulfoton sulfoxide was degraded at a GC injection port, and that a programmed temperature vaporization (PTV) inlet in the pulsed splitless mode reduced the degradation. Ueno *et al.*[20] reported that oxydemeton-methyl (sulfoxide of demeton-S-methyl) also decomposed at a GC injection port, and that demeton-S-methyl was easily oxidized to oxydemeton-methyl in the sample preparation. They thus added both L-ascorbic acid and butylhydroxytoluene as an antioxidant. Mastovska *et al.*[21] also reported on the thermodegradation of some sulfoxides. They said that the analyses of sulfoxides were difficult because of their unstable behavior in GC analyses.

Here, the problem with the concerned sulfoxides is derived from deoxdation in an EI ion source, was found. With a decrease in the concentration, the ratio of the “shifted base peak” became higher. This becomes a problem in the measurement corresponding to the water supply law. To meet the legal regulations, the pesticides have to be analyzed at ppb levels, and reproducible experimental data of the mass spectra is necessary.

By the way, a matrix-induced enhancement effect is often observed in the pesticide

residue analysis by GC or GC-MS.[3)-13)] This phenomenon means that the response of a pesticide in the matrix solution is higher than that in the matrix-free standard solution. It was reported that the matrix protected the analytes from adsorption or alternation during transfer from the injector to the column.[3)-5), 8),10)] In order to compensate for the matrix-induced enhancement effect, the priming injection technique by real samples,[22)] the standard addition technique, the matrix matching technique, or using a pseudo matrix, such as polyethylene glycol 300 (PEG 300),[23)] or analyte protectants,[24)-26)] are often used.

In the present work, the effect of PEG 300 on the GC-MS behavior of the sulfoxides was examined, and that PEG 300 prevented deoxidation in an EI ion source and a dirty injection port was found.

1.2 Experimental

1.2.1 Reagents and chemicals

The standards for pesticides with a purity of 98% or higher, except for fenthion sulfoxide (94.7%), were obtained from Hayashi Pure Chemical Ind., Ltd. (Osaka, Japan). The chemical structures of sulfide, sulfoxide and sulfone are shown in Fig.1.1. Acetone, dichloromethane, *n*-hexane and ethyl acetate were high-purity solvents for pesticide and PCB analysis, obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol, HPLC grade, and polyethylene glycol 300 (PEG 300), special grade, were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Bottled water was “Natural water of Minami-alps” from Suntory Holdings., Ltd. (Osaka, Japan).

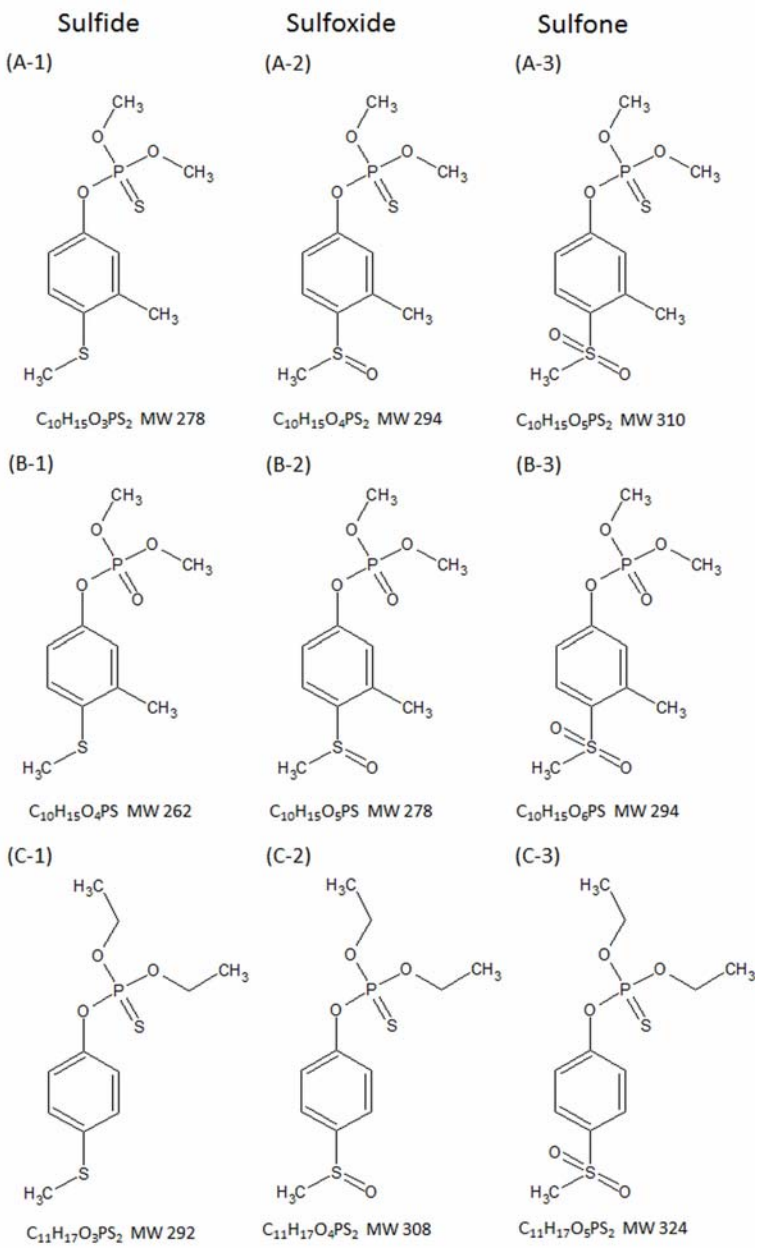


Fig. 1.1 Chemical structures of sulfide, sulfoxide and sulfone.

(A-1) fenthion, (A-2) fenthion sulfoxide, (A-3) fenthion sulfone,

(B-1) fenthion oxon, (B-2) fenthion oxon sulfoxide, (B-3) fenthion oxon sulfone,

(C-1) fensulfothion sulfide, (C-2) fensulfothion, and (C-3) fensulfothion sulfone.

1.2.2 Apparatus

GC-MS measurements were performed on an Agilent 7890A GC system coupled to a 5975C TAD mass spectrometer (Little Falls, DE, USA). GC-MS/MS analyses were performed on an Agilent 7890A GC system coupled to a 7000B triple quadrupole mass spectrometer (Santa Clara, CA, USA). Both systems were equipped with a 7693 autoinjector (Little Falls, DE, USA). The inlet temperature was 250°C, the total flow was set at 50 mL/min, and a split valve was opened 1.0 min after pulsed splitless injection (25 psi). The injection volume was 2 µL. A fused silica capillary column, HP-5msUI (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Agilent, Folsom, CA, USA) was used. At the beginning of injection, the oven temperature was set at 60°C for 1.0 min, ramped to 310°C at 20°C/min and then held for 3.0 min. The helium carrier gas flow rate was constant at 1.0 mL/min, and the transfer line temperature was set at 280°C. GC-MS was operated in a scan or SIM mode (SIM was for ppb level analyses), and GC-MS/MS was operated in the product ion scan mode. The source temperature was 230°C in both systems. As for the GC-MS/MS, nitrogen gas was used as the collision gas.

All data from Chemstation were converted to MassHunter software.

1.3 Results and Discussion

1.3.1 Concentration and solvent

The mass spectrum of each sulfoxide standard solution at 10 ppm in acetone was almost the same as that in the NIST library. The base peaks for fenthion sulfoxide, fenthion oxon sulfoxide, and fensulfothion were *m/z* 279, 263 and 293, respectively. However, the base peaks of sulfoxide at 1 ppm were shifted to a lower level by 1 *m/z*. The “shifted base peaks” of fenthion sulfoxide, fenthion oxon sulfoxide and

fensulfothion were *m/z* 278, 262 and 292, respectively (Fig. 1.2). The concordance of the retention time at both concentrations showed that the change in these spectra occurred neither in the GC injection port nor in the column. Furthermore, we tested various solvents, such as acetone, dichloromethane, *n*-hexane and ethyl acetate which are common in pesticide analyses, but there was no significant difference. This means that the changes in the spectra were occurred in an EI ion source. On the other hand, sulfones were stable, and no base peaks shift was observed.

1.3.2 Ion source temperature

Considering that the spectral change occurred in the ion source, as described above, we observed the spectra at various temperatures of the ion source. The results showed that the lower was the ion source temperature, the higher was the ratio of the "usual base peak". Nevertheless, the spectra were different from those in the NIST library even at low temperature. In addition, the shape of the "shifted base peak" at 150°C showed asymmetry, and the sensitivity was much lower than that at 230°C. Based on these results, the spectra change was caused by the interaction between sulfoxides and the EI ion source. However, over a 200°C ion source temperature is practical for environmental and food safety analyses in order to prevent any loss of sensitivity due to possible accumulated contamination from the sample matrix.

1.3.3 The cause of the "shifted base peak"

Demethylation is one of the most basic EI fragmentations, and it has high probability based on the chemical structure of the target sulfoxides. Therefore, the "usual base peak" ($[M-15]^+$) was reasonable, and the result of the product ion scan of the molecular ion supported this. (Fig 1.3, upper, example: fenthion sulfoxide). On the

(A)

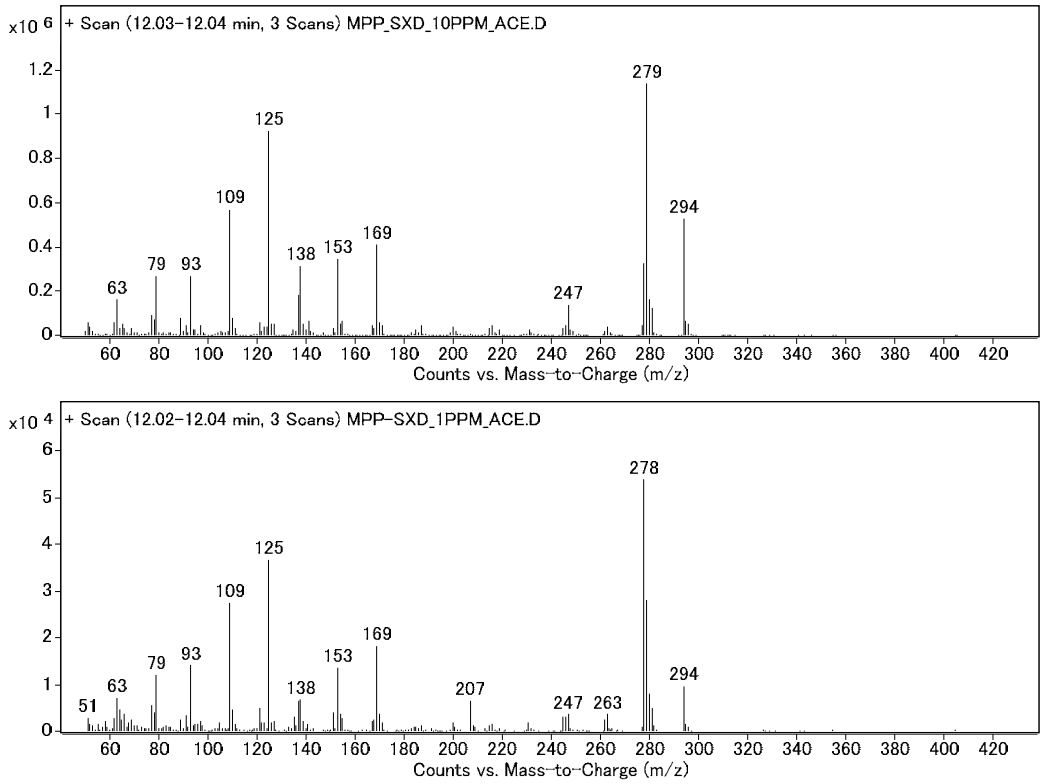


Fig. 1.2 (A) Spectra of fenthion sulfoxide.

Upper: 10 ppm, Lower: 1 ppm.

(B)

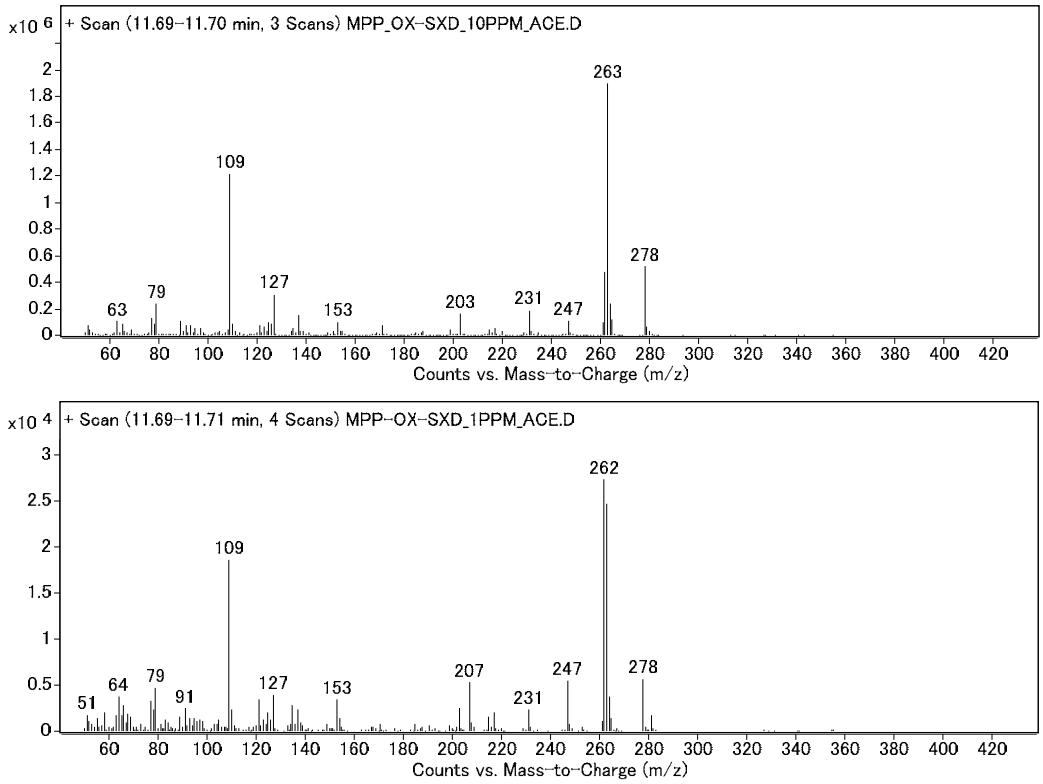


Fig. 1.2 (B) Spectra of fenthion oxon sulfoxide.

Upper: 10 ppm, Lower: 1 ppm.

(C)

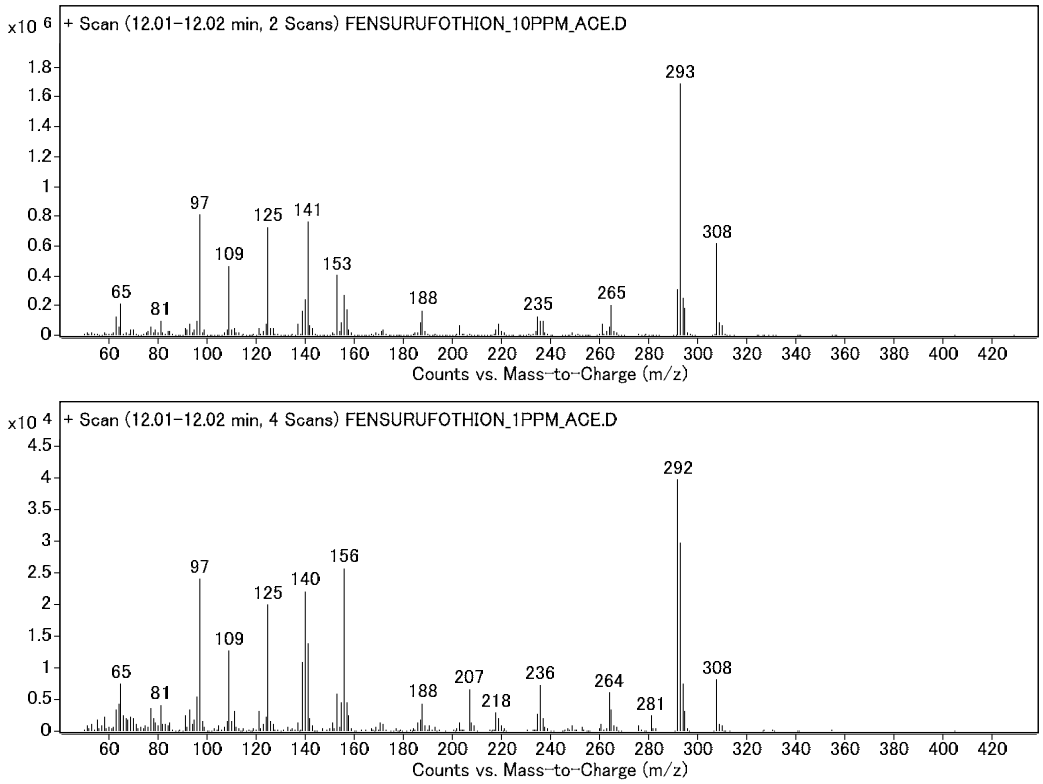


Fig. 1.2 (C) Spectra of fensulfothion.

Upper: 10 ppm, Lower: 1 ppm.

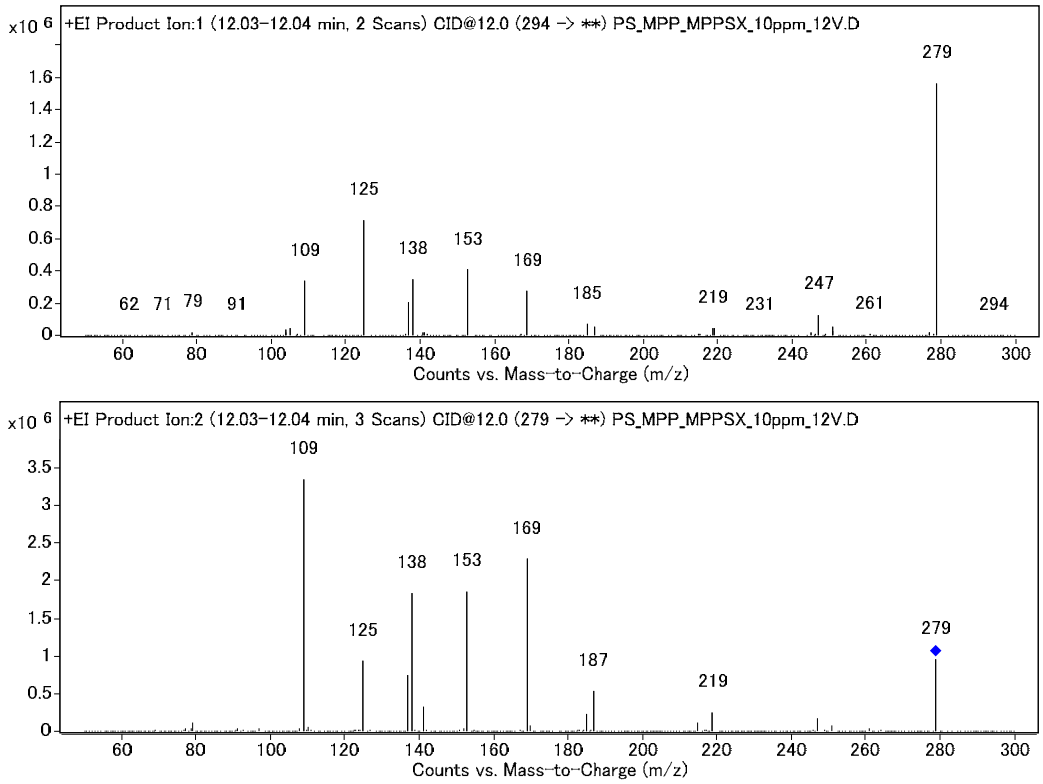


Fig. 1.3 Product ion scan spectra of fenthion sulfoxide.

Upper: precursor ion = molecular ion, *m/z* 294.

Lower, precursor ion = "usual base peak", *m/z* 279.

other hand, it is thought that the “shifted base peak” ($[M-16]^+$) was not formed by the common EI fragmentation. The result that the “shifted base peak” ($[M-16]^+$) was detected neither from the product ion scan of the molecular ion nor from that of the “usual base peak” ion supported, too. Although EI fragmentation and collision induced dissociation (CID) are different mechanisms, both are derive from the structure or functional group of the compounds. The example result of fenthion sulfoxide is shown in Fig. 1.3. After performing a product ion scan for the “shifted base peak”, the spectra were equal to the product ion scan of their sulfides. (Fig. 1.4). However, the product ion scan spectrum of fensulfothion could not be compared with that of fensulfothion sulfide, because fensulfothion sulfide could not be obtained. In consideration of these results and the similarity of the basic framework of sulfoxide and sulfide, it was concluded that the “shifted base peak” was formed from the sulfide, which was produced from the sulfoxide through deoxidation.

1.3.4 Addition of polyethylene glycol 300 (PEG 300)

Polyethylene glycol 300 (PEG 300), which has been commonly used to compensate for the matrix-induced enhancement effect, was used against this problem. The average molecular weight of PEG300 is 300 with a range of approximately 285 to 315, which covered the molecular weight and retention times of these three target sulfoxides. The added amount of PEG 300 was 250 ppm, and there was no memory of PEG 300 to the system (ion source, column and injection port) from its concentration and molecular weight. Since the ion source was temporarily coated, or PEG 300 was preferentially ionized, sulfoxides were avoided to direct contact with the metal surface. As a result, the formation of sulfide from sulfoxide was controlled and provided reproducible experimental data of mass spectra, even at low concentration (Fig. 1.5).

(A)

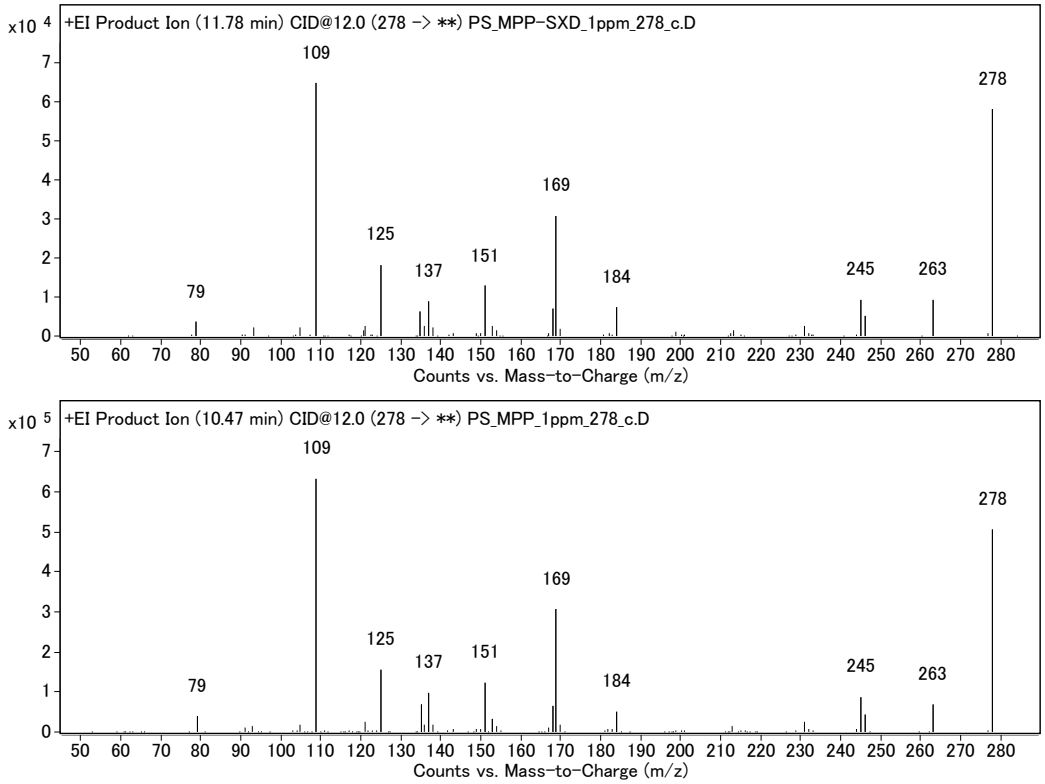


Fig. 1.4 (A) Product ion scan spectra of the "shifted base peaks" of sulfoxide and those of molecular ions of their sulfides.

Upper: fenthion sulfoxide (precursor ion = "shifted base peak" *m/z* 278)

Lower: fenthion (precursor ion = molecular ion *m/z* 278).

(B)

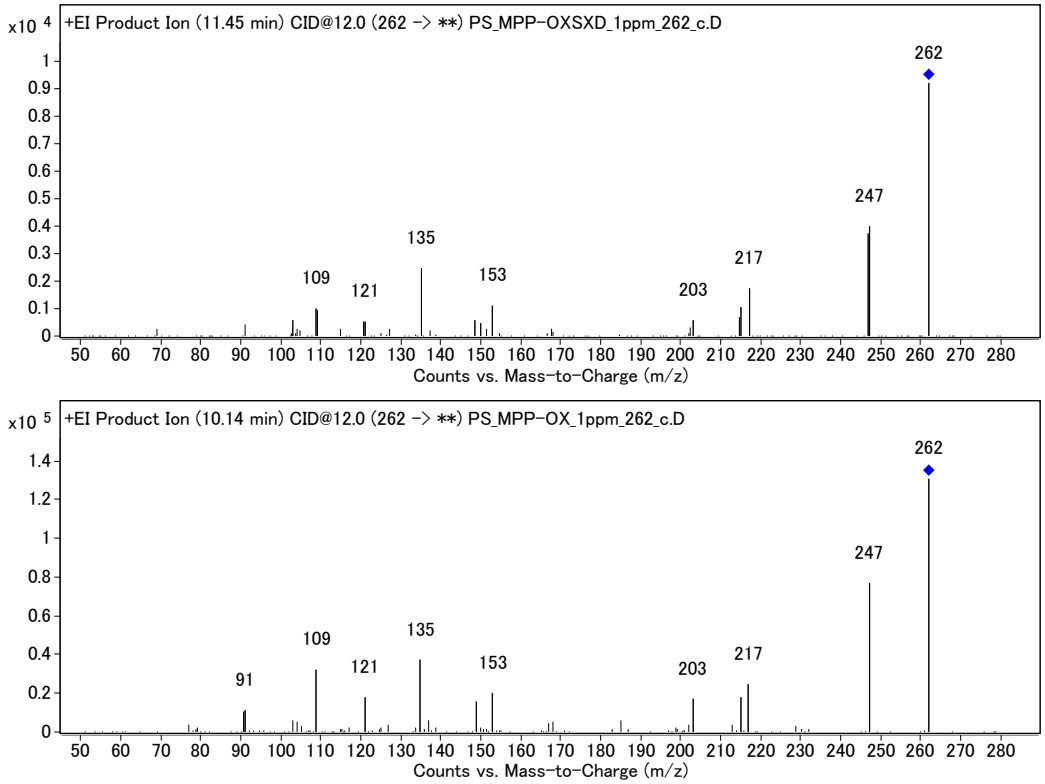


Fig. 1.4 (B) Product ion scan spectra of the "shifted base peaks" of sulfoxide and those of molecular ions of their sulfides.

Upper: fenthion oxon sulfoxide (precursor ion = "shifted base peak" *m/z* 262)

Lower: fenthion oxon (precursor ion = molecular ion *m/z* 262).

(A)

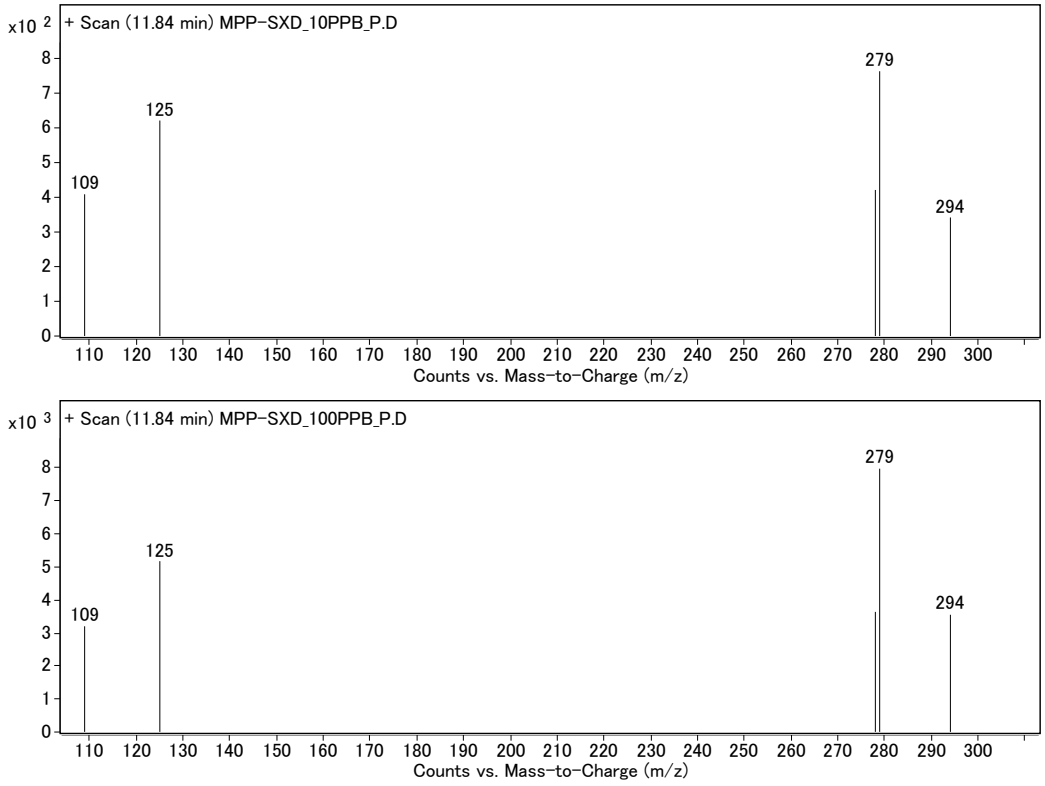


Fig. 1.5 (A) SIM spectra of fenthion sulfoxide at low levels with PEG300.

Upper: 10 ppb, Lower: 100 ppb.

(B)

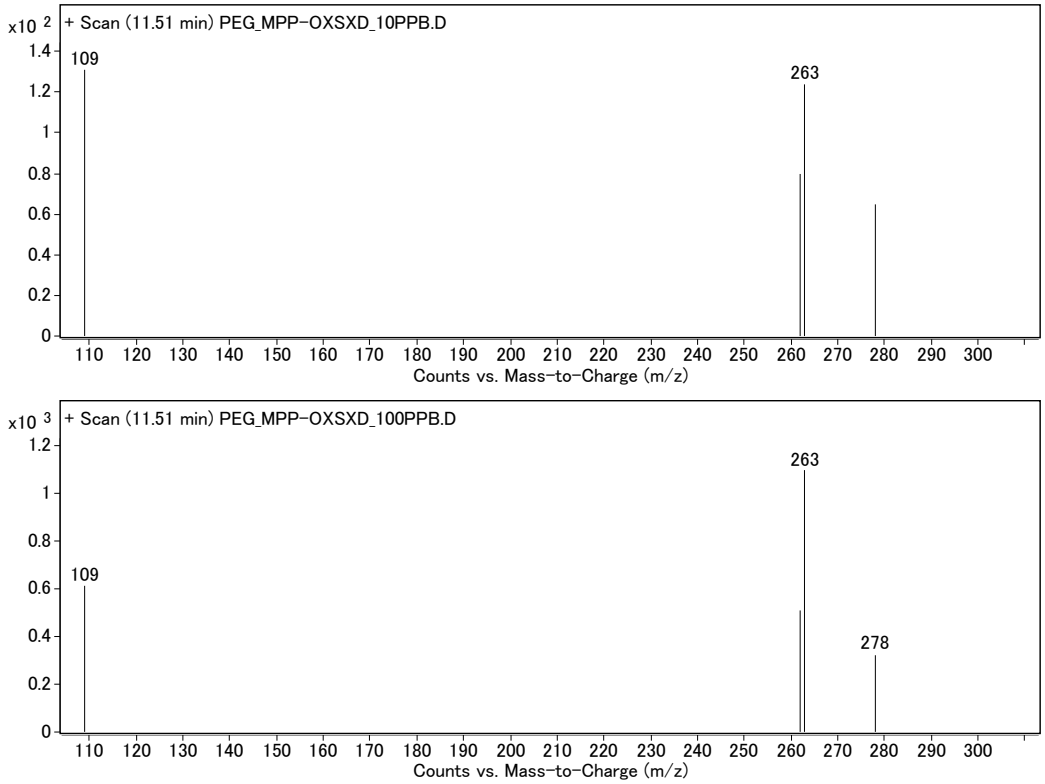


Fig. 1.5 (B) SIM spectra of fenthion oxon sulfoxide at low levels with PEG300.

Upper: 10 ppb, Lower: 100 ppb.

(C)

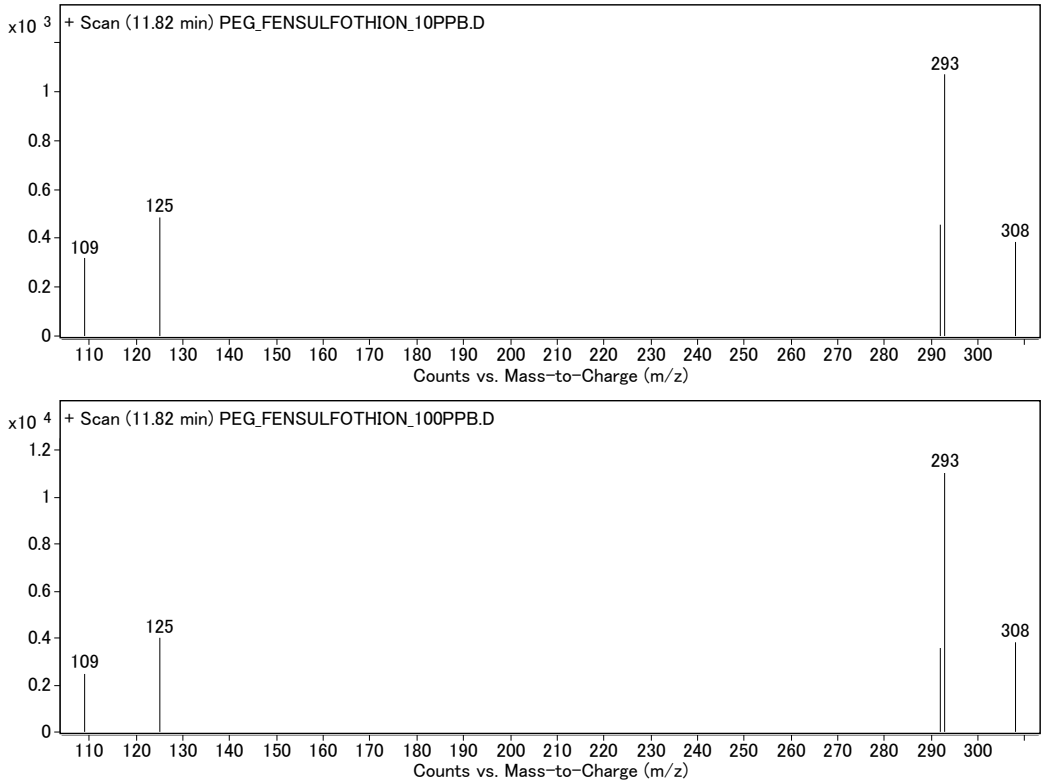


Fig. 1.5 (C) SIM spectra of fensulfothion at low levels with PEG300.

Upper: 10 ppb, Lower: 100 ppb.

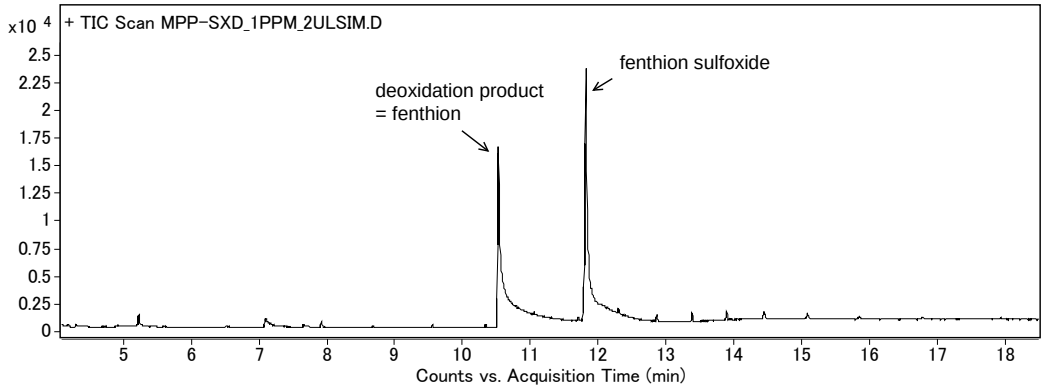
1.3.5 Possibility of deoxidation at the injection port

The plural GC-MS and GC-MS/MS for this test was used, and that the deoxidation of sulfoxides normally occured at the ion source was found. However, it was found that deoxidation could occur at the GC injection port, especially when the metal part at the bottom of the injection port was dirty. This phenomenon was observed after one hundred injections of derivatizing reagents (methoxyamine hydrochloride in pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane for another test) in the split injection mode. The liner and the column were renewed after the derivatized compound analysis, but the metal part which became rusty by the derivatization regant, was not exchanged. When deoxidation occurred at the injection port, two different peaks appeared. As for fenthion sulfoxide, one eluted at the fenthion sulfoxide retention time, and the other eluted at the fenthion retention time (Fig. 1.6). Fenthion oxon sulfoxide also showed two different peaks: one was fenthion oxon sulfoxide and the other was the deoxidized compound, fention oxon. By adding PEG300, the sulfide peaks was not produced and only sulfoxide peaks were obtained. This result indicates that PEG 300 prevented the deoxidation of sulfoxides at the injection port when using an extremely dirty metal part.

1.3.6 Additional effect

Since PEG300 has been used to compensate for the matrix-induced enhancement effect, we tested PEG 300 for other fenthion derivatives including fenthion. The target compounds were fenthion, fenthion oxon, fenthion sulfone, fenthion oxon sulfone, fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion. According to sample preparation reported by the Japanese Ministry of Health, Labour and Welfare,[1)] bottled water was extracted by a solid-phase extraction (SPE) column packed with polystyrene

(A)



(B)

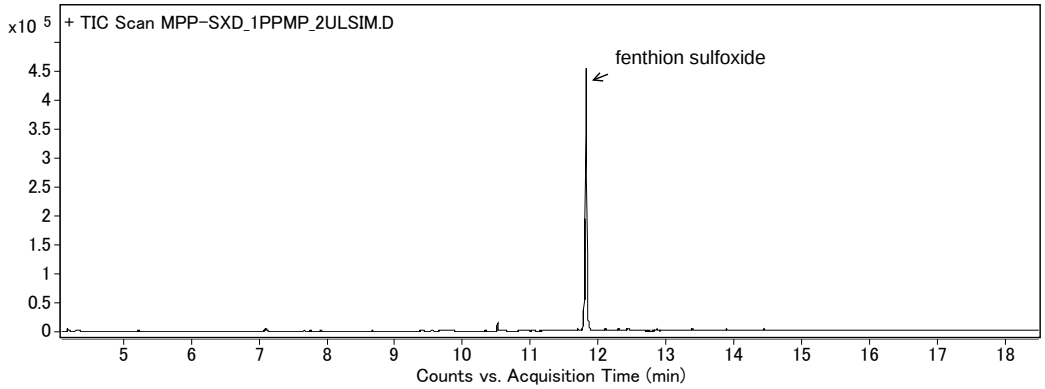


Fig. 1.6 Chromatogram of fenthion sulfoxide using an extremely dirty metal part at the bottom of the GC injection port.

(A) without PEG 300, (B) with PEG 300.

divinylbenzene (PS-DVB, 500 mg). The PS-DVB column was conditioned with dichloromethane, methanol and water in succession. Five hundred milliliters of water was loaded onto the PS-DVB column and eluted with 3 ml of dichloromethane. The eluted solution was concentrated with a stream of nitrogen gas, and the volume was adjusted to 1 ml. Fenthion and related compounds were fortified at 100 ppb level into the test solution. As a result, the matrix-induced enhancement effect values of these 7 pesticides in the eluted solution were between 141 and 703% without PEG 300, while, those were between 107-118% with PEG 300 (Table 1.1). PEG 300 not only prevented the sulfoxides deoxidation and provided reproducible experimental data of mass spectra, but also compensated for the matrix-induced enhancement effect.

1.3.7 Conclusions

Fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion were deoxidated at the EI ion source in the GC-MS system. The degrees of deoxidation were different depending on their concentrations, and had an influence on quantitative analysis at the ppb level. Adding PEG 300 prevented sulfoxide deoxidation was found. Also the deoxidated peaks at their sulfides retention time was observed when sulfoxides were injected into the injection port, which the metal part at the bottom of the injector port was extremely dirty. The addition of PEG300 was also effective in this case. Moreover, PEG 300 compensated for the matrix-induced enhancement effect for other fenthion derivatives, such as fenthion oxon, and fenthion sulfone.

Fig. 1.7 is a view showing a frame format of PEG 300 protecting sulfoxides interaction to the ion source.

Table 1.1 Matrix-induced enhancement effect value of each pesticide with and without PEG 300.

Compound	Monitor ion	Relative response, % [a]	
	(m/z)	Without PEG 300	With PEG 300
Fenthion	278	141	110
Fenthion oxon	262	161	107
Fenthion sulfone	310	170	117
Fenthion oxon sulfone	294	396	118
Fenthion sulfoxide	279	517	112
Fenthion oxon sulfoxide	263	544	112
Fensulfothion	293	187	113
[b] Fenthion sulfoxide	278	199	111
[b] Fenthion oxon sulfoxide	262	703	84
[b] Fensulfothion	292	167	99

a. Relative response of the analyte in the sample solution to that of the matrix-free standard solution.

b. Deoxidated ion (= “shifted base peak”).

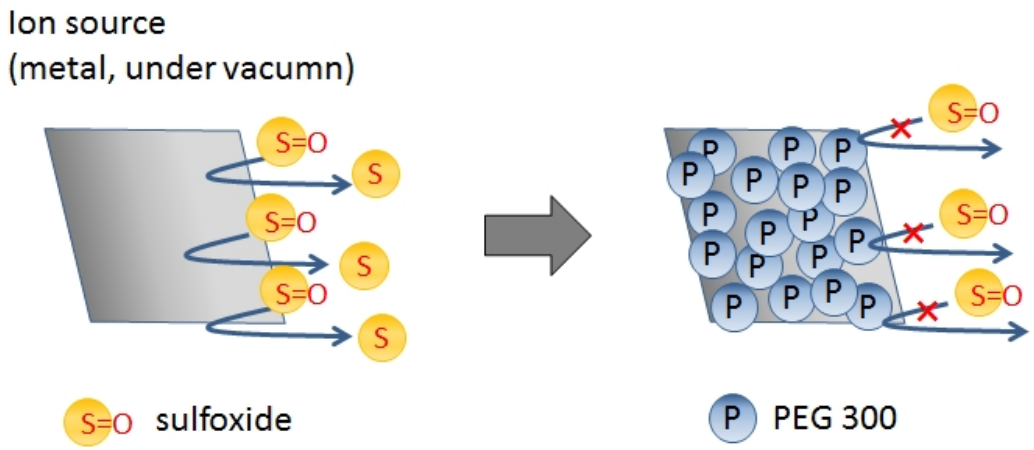


Fig. 1.7 Protection sulfoxides interaction to the ion source by PEG 300.

Chapter 2

Decrease in the Matrix Enhancement Effect of GC-MS by a Gold-Plated Ion Source

2.1 Introduction

GC-MS has been commonly used for pesticide multi-residue analyses since 2005. In Japan, the PLS has been in force since 2006,[28] and GC-MS has been used instead of 3 types of GC detectors (FPD, ECD, and NPD).[2] However, the matrix effect of pesticides analyzed in foods by GC/MS, a phenomenon in which the response of a pesticide is higher in the food matrix than that in the standard solution, has been often observed.[3-13] In order to calculate the exact quantity value, the priming injection technique by samples,[22] the standard addition technique, the matrix matching technique, a pseudo matrix, such as PEG 300,[23] and analyte protectants[24-26] are often used. However, matrix-free organic solvents are commonly used in pesticide analyses because of the abundance of samples.

The matrix effect on GC-MS has been suspected to occur at the injection port, the column, and the ion source, where matrix components pass through. The injection port was the most likely place[3,7] for the matrix effect to occur when GC detectors were widely used. In GC-MS, it is difficult to find the place where the matrix effect occurs, because the injection port, the column, and the ion source are connected to the vacuum system. It is also known that the presence of an inert surface in the system, sample clean-up, and the concentration rate of the samples influence the matrix effect.[7]

In this study, a gold-plated ion source was created that was more inert than the original ion source. Gold plating is a deactivated technology for use with the Agilent quadrupole. Many deactivated techniques are available, with patents from their manufactures, e.g., the solid inert ion source (Agilent, Little Falls, DE, USA), Silchrom

(Agilent), and Silicosteel (GL Science, Saitama, Japan). The gold plating technique was chosen because gold is the most inactive metal. D'Autry *et al*. demonstrated the stability of volatile compounds, such as methanol and ethanol, using headspace GC/MS with a gold-coated ion source.[28)] The most inert liner and column were chosen and the matrix effect was evaluated by the difference in the ion source.

2.2 Experimental

2.2.1 Reagents and chemicals

The pesticide mixture standards, PL-2-1 and PL-3-1 (listed in Table 2.1) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The pesticides mixtures are listed in Table 1. Acetone and *n*-hexane, high-purity solvents for the pesticide and PCB analyses, were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2.2 Analytical method

The Siltek deactivated injection liner (Restek, Bellefonte, PA, USA) and factorFOUR VF-5ms (Agilent, Middleburg, Netherlands) were chosen to establish the most inert system available. An Agilent 7890A gas chromatograph with a 5975C TAD mass spectrometer with a solid inert ion source and a 7693A autoinjector (Little Falls, DE, USA) was used. The operating conditions were as follows: injection liner, Siltek deactivated injection liner; column, factorFOUR VF-5ms, 30 m × 0.25 mm id, 0.25 μm thickness; oven temperature program, 70°C (1 min hold) to 125°C at 25°C/min and to 300°C at 10°C/min (10 min hold); injection temperature, 250°C; injection mode, pulsed splitless (30 psi, 1 min, 2 μL injection); carrier gas, He 1.0 ml/min constant flow; transfer line, 280°C; ion source temperature, 300°C.

2.2.3 Gold-plated ion source

A metalwork company in Japan was hired to gold-plate an existing ion source (a solid inert ion source). These ion source parts were electroplated. The gold material contained 99.7% Au and 0.3% Co, and Ni was used as the adhesive. Not only the ion source body but also the draw-out plate lens and the interface socket were coated with gold. The ion source body and the draw-out plate lens create an ionization place where an interaction could occur. The entrance lens, ion focus lens, and repeller were not coated this time because there was a possibility that the electric field might change. The plated thickness was 1.2-1.4 μm (Fig. 2.1).

2.1.4 Sample and sample preparation

Potato, spinach, orange, brown rice, and soybean were chosen as representative samples. They were prepared using a method that conformed to the "Multiresidue Method for Agricultural Chemicals by GC-MS (Agricultural Products)" for the PLS by the MHLW.[2)]

For fruits and vegetables, 20 g samples were weighted. For brown rice and soybean, 10 g samples were weighted and added to 20 mL of water, and then the mixture was stored for 15 min. After shaking with 50 mL of acetonitrile for 30 min, the samples were filtered. Acetonitrile was added to the samples to make 100 mL, and then 20 mL of the sample solution was measured (for brown rice and soybean: 40 mL). After 10 g of sodium chloride and 20 mL (brown rice and soybean: 40 mL) of a 0.5 M phosphate buffer (pH 7.0) were added, the samples were shaken for 10 min. A graphite carbon/aminopropyl silanized silica gel minicolumn (GCB/NH_2, 500 mg/500 mg, 20 mL) (Supelco, Bellefonte, PA, USA) was conditioned with 10 mL of acetonitrile/toluene (3:1). The sample solution was applied to the column and eluted

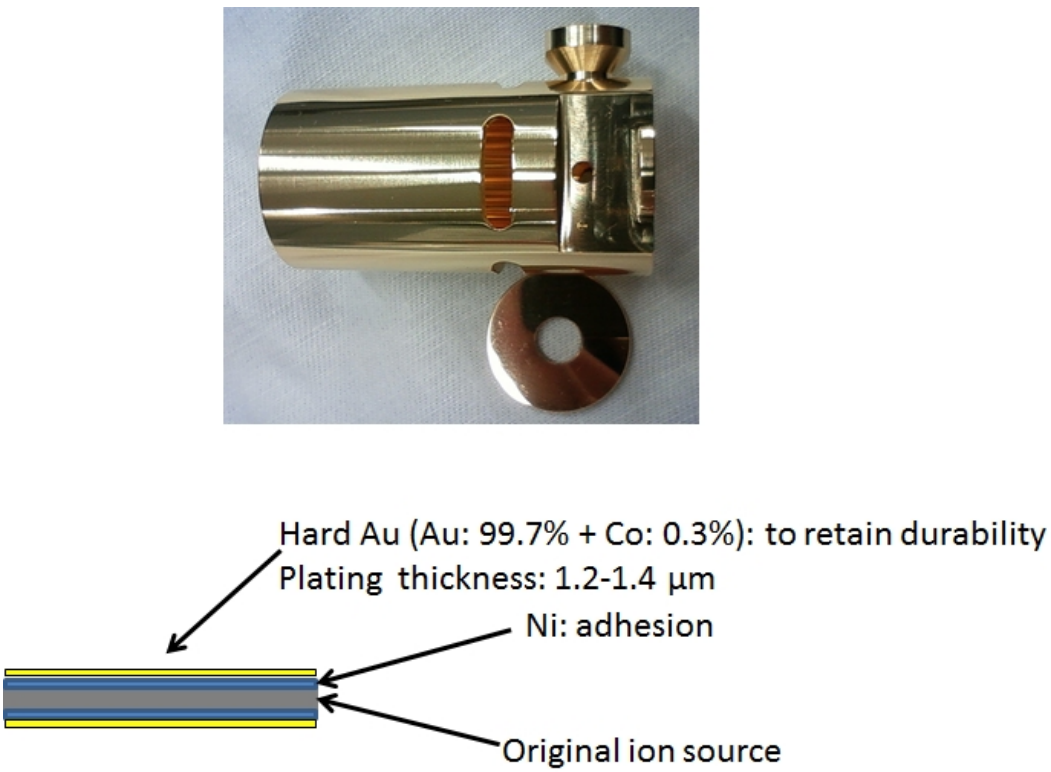


Fig. 2.1 Gold-plated ion source. Upper: picture of the ion source body with the interface socket and draw-out lens; lower: cross-section diagram.

with 20 mL of acetonitrile/toluene (3:1). The eluate was evaporated to dryness and the residue was dissolved in acetone/*n*-hexane (1:1) to make a 5 mL solution.

As for the samples of brown rice and soybean, an octadecylsilanized silica gel column (C18, 1 g, 6 mL) (Agilent, Lake Forest, CA, USA) was treated before the GCB/NH_2 column clean-up. The C18 column was conditioned with 10 mL of acetonitrile. The sample solution was applied to the column and eluted with 2 mL of acetonitrile.

The GC-MS measurement was performed in the following order:

pesticide standard → fortified sample (n = 3, consecutively) → solvent

First, the pesticide standard mixture was analyzed. Then, the sample solution was analyzed three times consecutively. Before moving to the next sample, the solvent (acetone:*n*-hexane, 1:1, v/v) was injected 3 times to avoid the influence of the previous sample. After checking the intensity, stability, and peak shape of the next standard, the next sample solution was analyzed. The sample order was potato, spinach, orange, brown rice and soybean. First, the matrix effect was measured using the original ion source (the new ion source). Then, the ion source was changed to the gold-plated ion source. The injection liner was also changed to a new one, and the tip of the column was cut by about 30 cm. The matrix effect value of the pesticide in the sample solutions was analyzed in the SIM mode, and the relative response of the pesticide in the sample solutions to that of the standard solution was calculated.

2.3 Results and Discussion

The GC-MS analysis showed a matrix effect (the matrix effect value was almost over

120%) in almost all samples using the original ion source (Table 2.1). The mean matrix effect value of the pesticides in the samples of potato, spinach, orange, brown rice and soybean was 132%, 202%, 181%, 240%, and 151%, respectively. The compounds which showed a high matrix effect had hydroxyl (-OH; e.g., bromopropylate and bitertanol) or amino groups (R-NH-; e.g., simazine and propyzamide), azoles (-N=; e.g., bitertanol, fipronil, difenoconazole, and triadimenol) and organophosphorus (OPs) (P=O, P=S; e.g., profenofos, phosmet, and pyraclofos). This result agreed with the reports by Brunete *et al.*[29] and Poole.[12] Moreover, low-polar compounds, such as pyrethroids (e.g., cypermethrin and fenvarelate), showed a high matrix effect value. The retention time of any organic compounds by a non-polar or low-polar column is sorted by the boiling point and polarity;[29] thus, the compounds which had higher RTs tended to show a high matrix effect (Table 2.1). Brunete *et al.* demonstrated that the addition of not only analyte protectants for the polar compounds but also corn oil and olive oil for the low-polar compounds compensated for the matrix effect.[29]

The GC-MS analysis has an advantage in multi-residue analysis over GC using 3 types of detectors. However, many pesticides cannot be separated by a GC capillary column and are often interfered with by the matrix components because simple clean-up is used for multi-residue analyses. Recently, GC-MS/MS has become more common for multi-residue analyses because of its selectivity. However, the problem of the matrix effect still remains. Therefore, we created a gold-plated ion source to form a more inert GC-MS.

The efficiency of the gold-plated ion source was evaluated for 80 pesticide standards. The abundance of pesticides at 50 ppb was 1.3-2.5 times higher than that of the original ion source. Since the matrix effect is remarkable at low concentration, the calibration curve shows a quadric curve. The calibration curves of fenitrothion (5-100ppb, 5points)

Table 2.1 Matrix effect value of each pesticide with original ion source.

Compound	RT	Monitor ion, *m/z*	Relative response, % [a]									
			Potato		Spinach		Orange		Brownrice		Soybean	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Propoxur	9.61	152	114	10	162	19	153	19	169	32	129	21
Ethoprophos	9.94	158	118	9.4	150	13	146	14	146	20	123	16
Carbofuran	11.05	164	132	12	194	28	187	21	202	43	139	26
Simazine	11.10	201	117	7.3	134	6.8	143	6.5	131	9.5	119	11
Quintozene	11.35	237	106	3.6	132	15	132	16	144	27	111	16
r-BHC	11.45	219	106	5.6	120	5.2	121	6.3	121	7.7	108	8.9
Propyzamide	11.52	173	117	6.6	141	8.2	137	6.4	132	11	117	11
Diazinon	11.54	304	114	4.0	137	8.1	135	8.7	134	14	116	11
Tri-allate	11.96	268	112	5.2	126	4.9	132	5.3	125	7.2	112	10
Propanil	12.47	161	117	9.2	142	8.2	147	10	108	11	121	14
Vinclozoline	12.61	285	114	5.2	132	2.7	127	4.4	124	5.8	111	9.4
Alachlor	12.69	160	111	6.3	131	12	134	11	140	17	113	13
Parathion-methyl	12.69	263	111	8.2	154	25	144	19	176	38	121	21
Pirimifos-methyl	13.08	290	117	8.2	143	9.1	139	8.9	142	14	119	13
Fenitrothion	13.17	277	113	8.0	156	24	150	19	182	38	123	21
Metolachlor	13.44	162	115	6.6	139	8.0	131	8.6	152	10	116	12
Chlorpyriphos	13.47	314	111	7.9	141	11	139	10	151	10	117	14
Fenthion	13.56	278	117	7.1	135	6.5	135	6.3	137	5.9	117	10
Isophenphos oxon	13.60	229	150	18	244	36	264	38	351	69	241	50
Parathion	13.62	291	115	6.9	151	23	154	20	221	35	123	21
Triadimefon	13.69	208	113	7.6	136	8.5	136	8.7	152	8.5	117	13
Fipronil	14.14	367	117	12	154	17	152	17	238	40	128	22
Allethrin-1,2	14.25	123	147	16	161	14	141	14	287	53	128	17
Isophenphos	14.25	213	124	8.0	152	16	152	14	178	17	124	17
Chlorfenvinphos-Z	14.30	267	124	10	157	17	155	14	186	20	128	19
Allethrin-3,4	14.32	123	131	9.2	155	11	154	19	252	31	133	17
Triadimenol-1	14.50	112	134	11	- [b]	24	169	15	250	19	- [b]	30
Triadimenol-2	14.65	112	132	11	- [b]	19	170	15	205	16	139	20
Tetrachlorvinphos	14.83	329	122	11	181	21	166	18	245	25	141	24
Endosulfan-α	15.09	241	110	5.2	125	2.8	126	5.1	- [b]	- [b]	151	70
Flutolanil	15.14	173	122	9.1	183	15	164	16	229	2.5	137	18
Isoprothiolane	15.28	118	119	10	153	4.3	152	7.4	162	1.4	127	13

continued

Profenofos	15.34	337	120	9.5	172	19	166	18	270	10	137	23
Oxyfluorfen	15.48	252	116	6.7	164	20	160	19	308	27	132	22
Myclobutanil	15.50	179	122	5.2	126	2.8	145	7.5	154	11	119	13
Buprofezin	15.54	172	118	7.6	139	5.3	132	6.5	141	1.3	115	10
Cyproconazole	15.87	222	134	11	171	10	127	17	201	5.0	133	18
Chlorbenzilate	16.04	139	119	8.5	152	7.6	150	7.8	177	2.4	133	14
Ethion	16.18	231	147	11	203	21	184	18	239	9.1	144	20
Triazophos	16.48	257	148	12	201	24	184	20	255	14	141	25
Propiconazole-1	16.78	259	137	9.1	174	3.4	158	10	193	2.8	139	15
Quinoxyfen	16.84	237	117	6.6	150	3.9	143	5.4	149	0.9	126	10
Propiconazole-2	16.89	259	128	10	170	4.1	151	10	179	6.0	138	14
Hexazinone	17.04	171	134	8.5	190	3.1	178	8.7	188	3.1	151	16
Propargite	17.16	173	126	8.6	192	23	175	24	320	38	149	28
Tebuconazole	17.20	250	143	10	199	7.3	175	15	213	7.1	144	17
Acetamiprid	17.71	166	279	26	806	60	664	104	973	57	389	66
Phosmet	17.82	160	130	7.9	205	21	190	28	337	34	151	28
Bromopropylate	17.86	341	144	10	218	17	205	21	270	10	175	24
Fenpropathrin	17.94	181	134	9.0	202	14	171	16	222	9.1	140	20
Methoxychlor	17.95	227	130	8.4	189	14	170	18	252	13	145	21
Cyhalothrin-λ	18.51	181	143	10	209	18	189	22	292	23	152	25
Pyriproxyfen	18.58	136	134	7.8	166	4.0	182	8.8	163	1.8	125	12
Cyhalothrin-γ	18.69	181	141	6.3	201	15	179	21	275	24	157	25
Pyraclofos	19.23	360	153	13	318	30	268	36	420	28	221	38
Bitertanol-1	19.50	170	166	13	367	14	291	31	352	8.1	231	29
Bitertanol-2	19.61	170	163	12	429	20	313	37	425	12	270	34
Pyridaben	19.73	147	136	7.8	253	12	217	24	319	10	176	25
Fenbuconazole	19.74	340	124	7.2	182	4.1	166	13	188	2.8	136	14
Fluquinconazole	20.11	198	150	10	235	3.8	203	15	237	5.2	162	17
Cypermethrin	20.38-20.58	163	139	12	486	34	219	28	353	22	191	30
Fluridone	20.98	328	187	10	342	8.8	300	36	331	9.0	274	23
Fenvalerate-1	21.31	225	131	9.0	259	22	202	28	319	31	210	25
Fenvalerate-2	21.54	225	145	9.3	244	15	195	28	326	32	192	25
Difenoconazole-1	21.89	323	185	16	439	23	348	46	575	23	348	41
Difenoconazole-2	21.96	323	151	12	330	13	267	31	385	15	240	24
Deltamethrin	22.21	253	128	6.2	188	14	152	17	264	67	110	21
Mean			132		202		181		240		151	

[a)] Relative response of the pesticide in each sample solution to that of the standard solution, [b)] Matrix interfered.

using the gold-plated ion source were improved (Fig. 2.2). This is because the interaction of the pesticides and the ion source decreased.

The mean matrix effect value of the pesticides in the samples of potato, spinach, orange, brown rice, and soybean decreased by 2.5, 14, 20, 38, and 15%, respectively, using the gold-plated ion source (Table 2.2). The rate of decrease was significant in the sample of brown rice. The sample of brown rice contained many more matrices than those of other agricultural products,[31] and, when the gold-plated ion source was used, the interaction between the matrices and the ion source was reduced. The result of some representative pesticides showed in Fig. 2.3.On the other hand, since the sample of potato had few matrices, its matrix effect was small, and, therefore, the rate of decrease in the matrix effect was also small. From these results, the amount of matrices influenced the matrix effect. The pesticides whose matrix effects decreased by using the gold-plated ion source also had hydroxyl or amino groups, azols, OPs, and pyrethroids. This might be because both the interaction between the matrices and the ion source and the adsorption or decomposition of pesticides was reduced by the gold-plated ion source. Fig. 2.4 is a view showing a frame format of interaction difference between pesticide/matrix and ion source. Meanwhile, the matrix effect value did not decrease in the pesticides having a heterocyclic amine structure, such as triadimefon, triazophos, hexadinone, acetamiprid, phosmet, pyraclofos, and fluquinconazole. These pesticides might be influenced by the injection port or the column rather than by the ion source. As for deltamethrin, isomerization at the injection port occurred.[21]

The cost of gold plating was under $100 per source. However, the robustness problem remains during maintenance. It is also necessary to approach this problem from the viewpoint of both entire GC-MS system and the sample preparation.

Nevertheless, although the matrix effect was not completely controlled by the

gold-plated ion source, the gold-plated ion source effectively reduced the matrix effect.

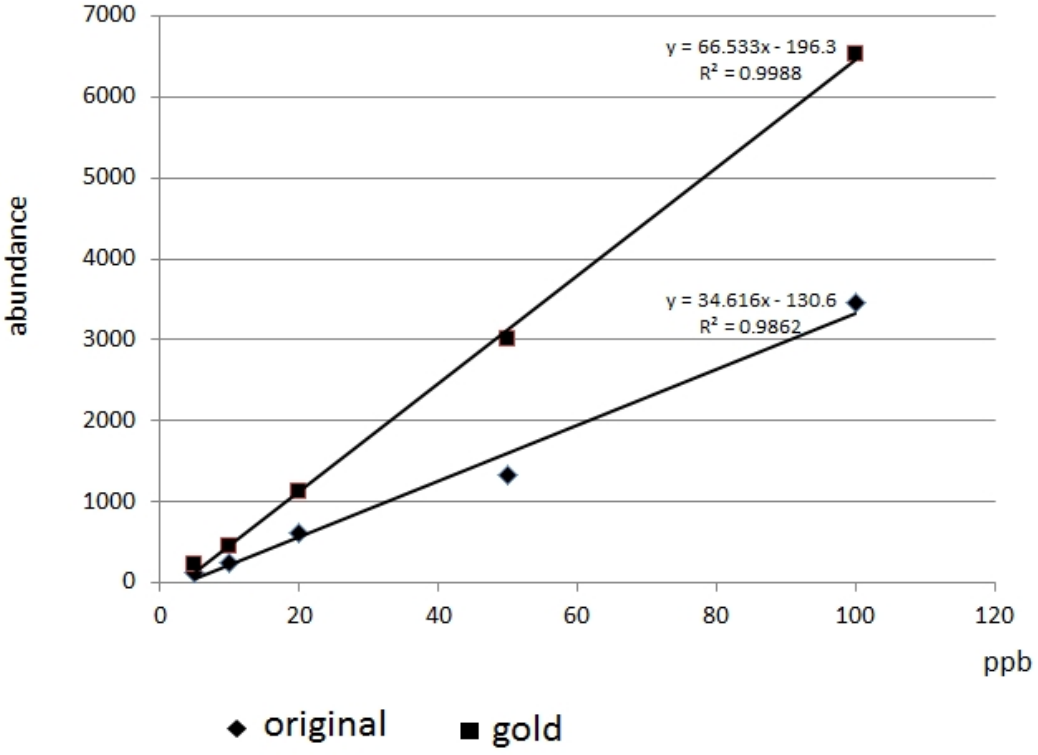
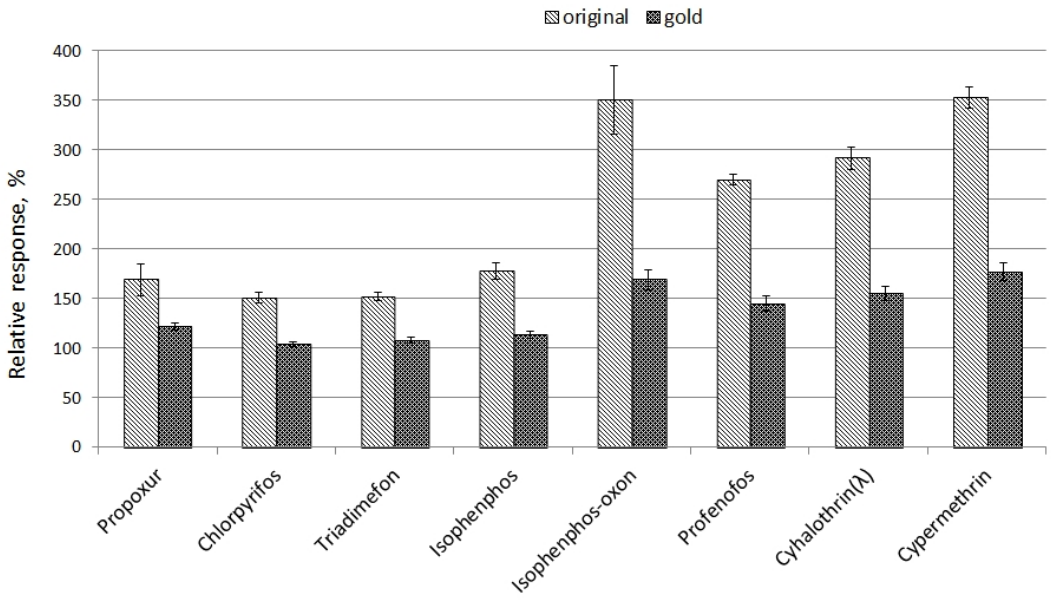


Fig. 2.2 Calibration curves of fenitrothion.



*Relative response (%) = Relative response of pesticide in brown rice solution to that of the matrix-free standard solution

Fig. 2.3 Comparison of matrix enhancement effect using original ion source and gold-plated ion source.

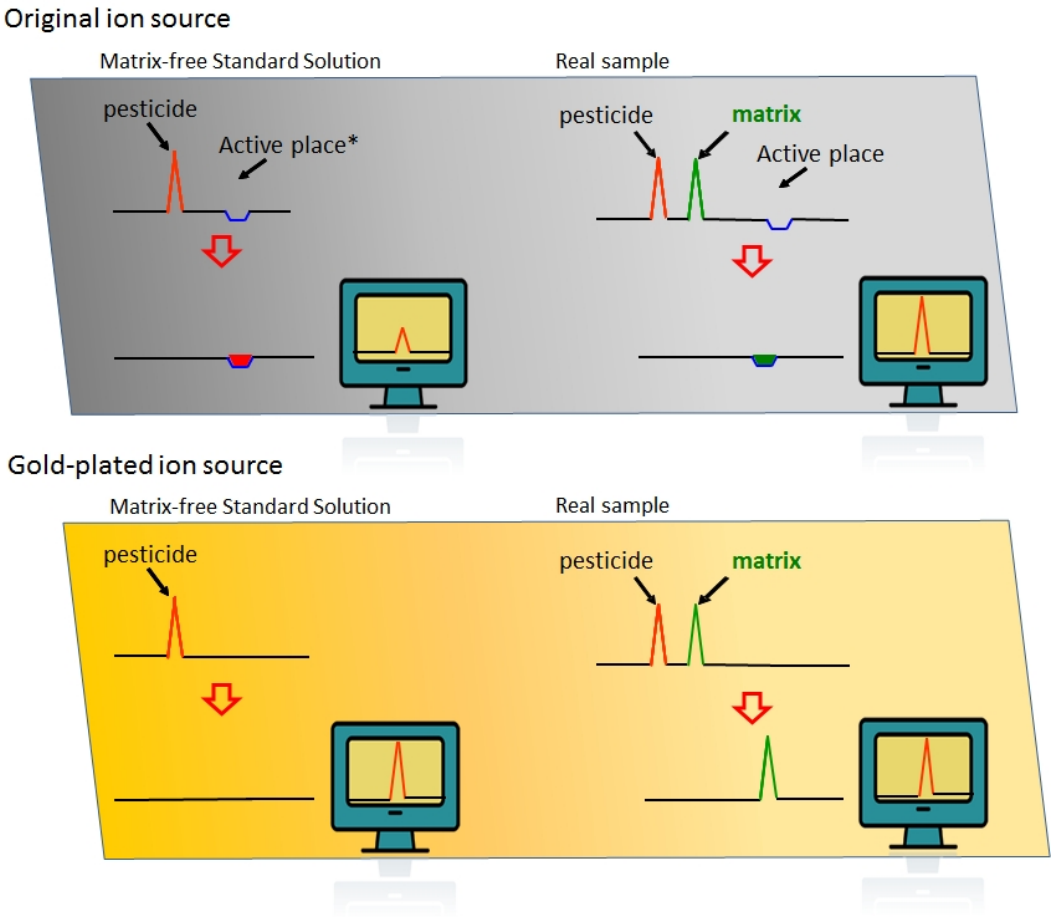


Fig. 2.4 Interaction difference between pesticide/matrix and ion source.

Table 2.2 Matrix effect value of each pesticide with gold-plated ion source.

Compound	RT	Monitor ion, *m/z*	Relative response, % [a]									
			Potato		Spinach		Orange		Brown rice		Soybean	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Propoxur	9.61	152	115	3.3	134	11	124	7.9	122	7.9	113	8.1
Ethoprophos	9.94	158	116	3.8	132	10	124	6.1	121	15	110	7.6
Carbofuran	11.05	164	137	5.0	161	13	145	7.8	131	16	123	8.9
Simazine	11.10	201	117	3.3	122	5.0	128	2.8	98.6	4.4	108	4.7
Quintozene	11.35	237	108	3.8	123	11	118	7.7	118	14.6	104	6.9
r-BHC	11.45	219	104	0.9	107	3.1	109	1.9	101	3.9	100	2.2
Propyzamide	11.52	173	114	2.6	122	4.9	121	4.0	105	5.1	111	6.8
Diazinon	11.54	304	115	3.1	121	5.2	119	5.0	104	5.2	108	4.3
Tri-allate	11.96	268	110	1.9	108	4.2	118	2.0	106	6.5	105	4.6
Propanil	12.47	161	120	5.3	131	6.7	132	5.1	114	9.1	110	9.5
Vinclozoline	12.61	285	111	2.1	116	5.2	116	2.2	97	0.7	105	5.2
Alachlor	12.69	160	110	2.6	120	5.8	119	5.0	104	4.5	105	6.4
Parathion-methyl	12.69	263	113	5.0	137	17	122	13	135	24	113	14
Pirimifos-methyl	13.08	290	117	4.0	124	6.1	122	4.8	103	5.4	109	6.7
Fenitrothion	13.17	277	117	5.7	141	17	127	12	139	20	115	13
Metolachlor	13.44	162	114	3.3	122	5.7	119	3.9	116	4.2	108	6.7
Chlorpyriphos	13.47	314	113	5.0	116	5.9	121	4.7	104	4.3	104	7.4
Fenthion	13.56	278	116	4.1	119	4.9	122	4.1	105	3.7	107	6.5
Isophenphos oxon	13.60	229	140	9.5	178	19	173	17	169	20	154	22
Parathion	13.62	291	114	2.8	139	14	132	14	171	20	117	15
Triadimefon	13.69	208	115	2.1	120	5.4	122	5.2	108	6.0	107	81
Fipronil	14.14	367	130	7.8	151	12	137	9.3	153	13	122	14
Allethrin-1,2	14.25	123	132	6.2	105	5.3	120	5.5	211	35	129	14
Isophenphos	14.25	213	119	4.9	127	7.1	126	6.0	113	7.0	108	8.5
Chlorfenvinphos-Z	14.30	267	123	6.0	132	7.6	131	6.0	124	8.9	114	9.9
Allethrin-3,4	14.32	123	125	5.1	132	6.6	133	6.3	153	13	118	11
Triadimenol-1	14.50	112	133	7.2	- [c]	16	140	6.7	165	12	- [b]	17
Triadimenol-2	14.65	112	132	6.5	- [c]	13	138	6.8	137	11	120	11
Tetrachlorvinphos	14.83	329	124	7.4	148	9.4	134	6.9	139	9.3	121	12
Endosulfan-α	15.09	241	106	1.4	106	4.5	114	2.0	- [b]	- [b]	102	3.6
Flutolanil	15.14	173	125	8.3	157	9.0	140	5.3	141	9.5	199	17
Isoprothiolane	15.28	118	118	4.6	127	7.5	133	2.3	118	5.8	119	8.2

continued

Profenofos	15.34	337	126	9.5	152	9.9	140	8.5	145	13	121	11
Oxyfluorfen	15.48	252	110	5.9	148	14	143	14	209	17	133	18
Myclobutanil	15.50	179	119	5.3	125	15	130	4.7	110	18	110	9.4
Buprofezin	15.54	172	114	3.2	118	7.8	120	2.8	107	5.4	106	6.9
Cyproconazole	15.87	222	137	7.0	153	12	99	75	139	10	123	11
Chlorbenzilate	16.04	139	117	5.0	125	6.9	126	3.9	116	7.6	112	7.4
Ethion	16.18	231	140	8.4	174	13	153	7.9	151	12	127	12
Triazophos	16.48	257	139	13	187	19	151	10	154	16	119	13
Propiconazole-1	16.78	259	135	5.9	153	9.1	142	5.6	138	11	123	11
Quinoxyfen	16.84	237	118	5.2	128	9.0	131	2.0	112	8.6	116	6.9
Propiconazole-2	16.89	259	131	6.2	157	9.8	135	3.8	128	8.6	125	9.0
Hexazinone	17.04	171	132	5.9	152	9.3	148	3.3	135	9.1	134	9.5
Propargite	17.16	173	115	8.1	164	15	150	14	197	28	136	18
Tebuconazole	17.20	250	138	11	175	10	150	6.9	143	14	126	12
Acetamiprid	17.71	166	234	43	349	18	308	14	365	44	324	20
Phosmet	17.82	160	139	11	204	14	160	10	176	19	136	14
Bromopropylate	17.86	341	150	9.8	167	11	145	4.9	132	11	124	9.2
Fenpropathrin	17.94	181	133	7.7	156	10	136	3.5	122	9.7	115	9.1
Methoxychlor	17.95	227	125	7.9	170	11	142	8.8	140	14	117	9.6
Cyhalothrin-λ	18.51	181	154	10	191	12	148	7.0	155	15	129	11
Pyriproxyfen	18.58	136	132	6.8	142	10	161	3.8	126	12	120	8.9
Cyhalothrin-γ	18.69	181	143	9.1	185	11	151	6.4	157	15	131	12
Pyraclofos	19.23	360	159	18	294	19	206	13	232	25	178	17
Bitertanol-1	19.50	170	152	15	301	17	202	7.2	195	18	169	15
Bitertanol-2	19.61	170	145	14	311	18	206	8.9	208	21	179	16
Pyridaben	19.73	147	135	9.0	194	12	151	6.2	163	15	137	12
Fenbuconazole	19.74	340	126	8.3	161	11	141	4.1	127	12	101	26
Fluquinconazole	20.11	198	163	15	261	15	172	5.1	174	18	149	12
Cypermethrin	20.38-20.58	163	153	13	418	20	168	13	177	18	140	12
Fluridone	20.98	328	172	13	265	13	204	5.0	220	20	198	12
Fenvalerate-1	21.31	225	135	11	234	12	158	9.9	178	19	143	14
Fenvalerate-2	21.54	225	144	13	242	13	160	10	184	19	144	10
Difenoconazole-1	21.89	323	129	20	376	15	235	13	277	33	237	23
Difenoconazole-2	21.96	323	113	17	306	12	198	8.9	213	26	178	18
Deltamethrin	22.21	253	113	7.8	210	13	137	14	213	28	115	15
Mean			129		173		145		149		129	

[a)] Relative response of the analyte in each sample solution to that of the standard solution, [b)] Matrix interfered.

Chapter 3

Search of Components causing Matrix Enhancement Effect on GC-MS for Pesticide Analysis in Foods

3.1 Introduction

As mentioned in Chapter 2, the matrix enhancement effect occurs in any sample. However, the extent of which depends on the sample. In this chapter, what matrix components remained in the sample solution for GC-MS and what components caused the matrix effect, were studied. In order to identify the matrix components, the sample solution were derivatized to use metabolomics analysis technique. This technique is described in detail in Chapter 4.

Hereafter, the solution extracted and cleaned up from agricultural products was identified as a "sample solution." The solution consisting of single (or several) matrix component(s) was identified as a "matrix solution."

3.2 Experimental

3.2.1 Materials and Methods

3.2.1.1 Experiment 1: Search for the components in agricultural products

Potato, spinach, orange, brown rice, and soybean were chosen as representative samples. They were prepared by a method in conformity with the "Multiresidue Method for Agricultural Chemicals by GC/MS" for the PLS by the MHLW.[2)]

Methoxyamine hydrochloride and pyridine hydride were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Thermo Fisher Scientific (Rockford, IL, USA). Myristic acid-d_{27} was used as an internal standard and

purchased from Sigma-Aldrich (St Louis, MO, USA). Myristic acid-d_{27} was used to check whether delivatization was successful and as the compound for Retention Time Locking (RTL) for the Fiehn Metabolomics Library (Agilent Technologies, Inc.) to identify the components.[32)]

Applying the technique of metabolomics[33)] to the samples, comprehensive analyses of the matrices were studied. For derivatization, the sample solutions were dried by a centrifugal concentrator. The residues were then methoxyaminated with methoxyamine hydrochloride in pyridine (40 mg/ml) for 90 minutes at 30°C. For trimethylsilylation, MSTFA + 1% TMCS was added to the methoxyaminated samples, and the mixtures were stored for 30 minutes at 37°C. We also tested the sample solutions without derivatization.

3.2.1.2 Experiment 2: Evaluation of the components that cause the matrix enhancement effect

Pesticide mixture standards, PL-2-1 and PL-3-1 (listed in Table 1), were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tocopherols, sterols, phytol, and monoacylglycerols were chosen to evaluate the matrix effect. The representative components of the tocopherols and sterols were α-tocopherol and stigmasterol, respectively. The representative monoacylglycerols were 1-monomyristin, 1-monopalmitin, 1-monoolein, and 1-monostearin. α-Tocopherol (brand name: DL-α-tocopherol) and phytol were obtained from Wako Pure Chemical Industries (Osaka, Japan), and stigmasterol was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 1-monomyristin (brand name: *rac*-glycerol 1 myristate), 1-monopalmitin (brand name: DL-α-palmitin), 1-monoolein (brand name: 1-oleoyl-*rac*-glycerol), and 1-monostearin (brand name: stearoyl-*rac*-glycerol) were

purchased from Sigma-Aldrich. α-Tocopherol, phytol, and stigmasterol were dissolved in acetone/*n*-hexane (3:7, v/v) and prepared at 1, 10, 100, 200, 500, and 1000 ppm. The monoacylglycerol mixture (1:1:1:1, v/v) was prepared from 1 ppm to 500 ppm. The pesticide mixture was fortified to 100 ppb for each matrix solution.

3.2.1.3 Analytical method for Experiment 1

An Agilent 7890A gas chromatograph equipped with a 5975C TAD mass spectrometer and a 7693A autoinjector was used. The operating conditions were as follows: injection liner, Ultra inert liner for low pressure with glass wool (Agilent); column, DB-5ms + Duragurd*, 30 m × 0.25 mm id, 0.25 μm thickness, *nonpolar deactivated precolumn connected to DB-5ms, 10m (Agilent); oven temperature program, 60°C (1 min hold) to 325°C at 10°C/min (10 min hold); injection temperature, 250°C; injection mode, split (split ratio, 10:1); carrier gas, He 1.1 ml/min, constant flow; transfer line, 290°C; ion source temperature, 250°C.

3.2.1.4 Analytical methods for Experiments 2

An Agilent 7890A gas chromatograph equipped with a 5975C TAD mass spectrometer and a 7693A autoinjector (Little Falls, DE, USA) was used. The operating conditions were as follows: injection liner, Siltek deactivated liner (Restek, Bellefonte, PA, USA); column, factorFOUR VF-5ms, 30 m × 0.25 mm id, 0.25 μm thickness (Agilent Technologies, Middleburg, Netherlands); oven temperature program, 70°C (1 min hold) to 125°C at 25°C/min and to 300°C at 10°C/min (10 min hold); injection temperature, 250°C; injection mode, pulsed splitless (30 psi, 1 min); carrier gas, He 1.0 ml/min constant flow, transfer line, 280°C; ion source temperature, 300°C.

The GC/MS measurement was performed in the following order:

pesticide standard → matrix solution (n = 2, consecutively) → solvent

First, the pesticide standard mixture was analyzed. Then, the sample (or matrix) solution was analyzed three times (or twice) consecutively. Before moving to the next sample (or matrix) solution, the solvent (acetone:*n*-hexane, 1:1, v/v) was injected several times to avoid the influence of the previous sample. After checking the intensity, the stability, and peak shape of the next standard, the next sample (or matrix) solution was analyzed. The matrix effect value of the pesticide in each sample (or matrix) solution was evaluated as the relative response of the pesticides in the sample (or matrix) solution to that of the standard solution.

3.3 Results and Discussion

3.3.1 Experiment 1: Search for the components in agricultural products

Almost the same results was obtained with and without derivatization, but sensitivity was 2-5 times higher with derivatization. Furthermore, the reproducibility of the fatty acids was more stable in the different types of sample solutions.[34)] Using the Fiehn Metabolomics Library with the retention time information was also an advantage for delivatization. The derivatized chromatogram of each sample solution is shown in Fig. 3.1.

The components were identified by the NIST Mass Library and the Fiehn Metabolomics Library. Sterols remained in the extracts from the potato, spinach, and orange samples because the C18 column cleanup was not treated. The potato sample contained low matrix components compared to the other samples. The common matrix components remaining in the spinach, orange, brown, rice and soybean samples were

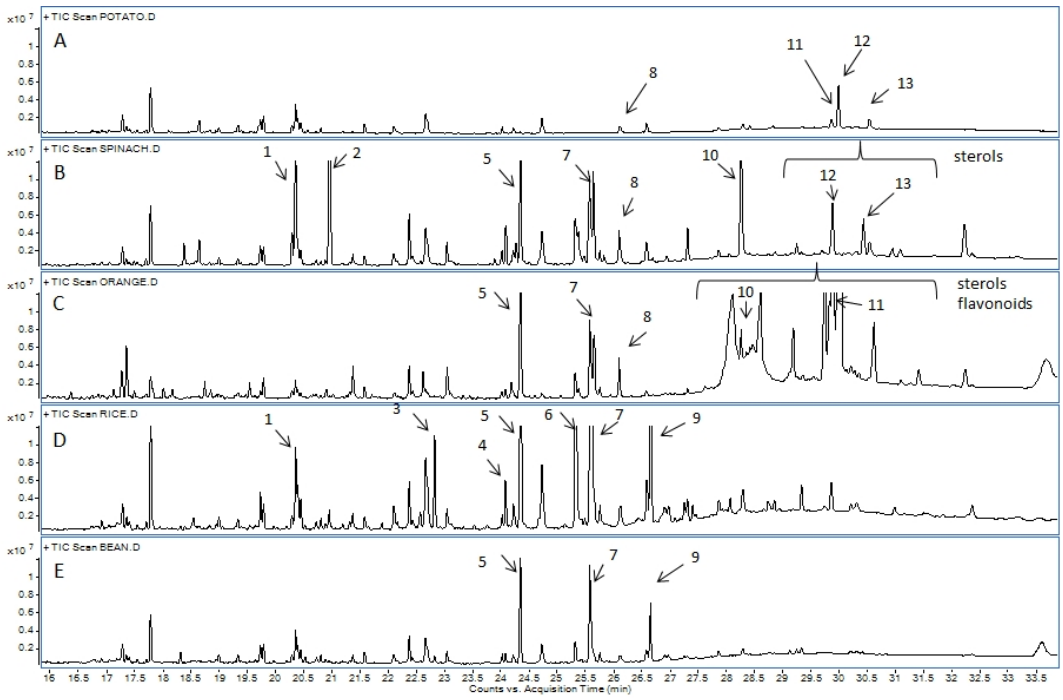


Fig. 3.1 Total ion chromatograms of derivatized samples with methoxyamine and MSTFA + 1 % TMCS in GC/MS analyses, A: potato, B: spinach, C: orange, D: brown rice, E: soybean, 1: linolenic acid methyl ester, 2: phytol, 3: 1-monomyristin, 4: 2-monopalmitin, 5: 1-monopalmitin, 6: 2-monolinolein, 7: 1-monolinolein, 8: squalene, 9: δ-tocopherol, 10: *α*-tocopherol, 11: β-sitosterol, 12: stigmasterol, 13: cycloartenol.

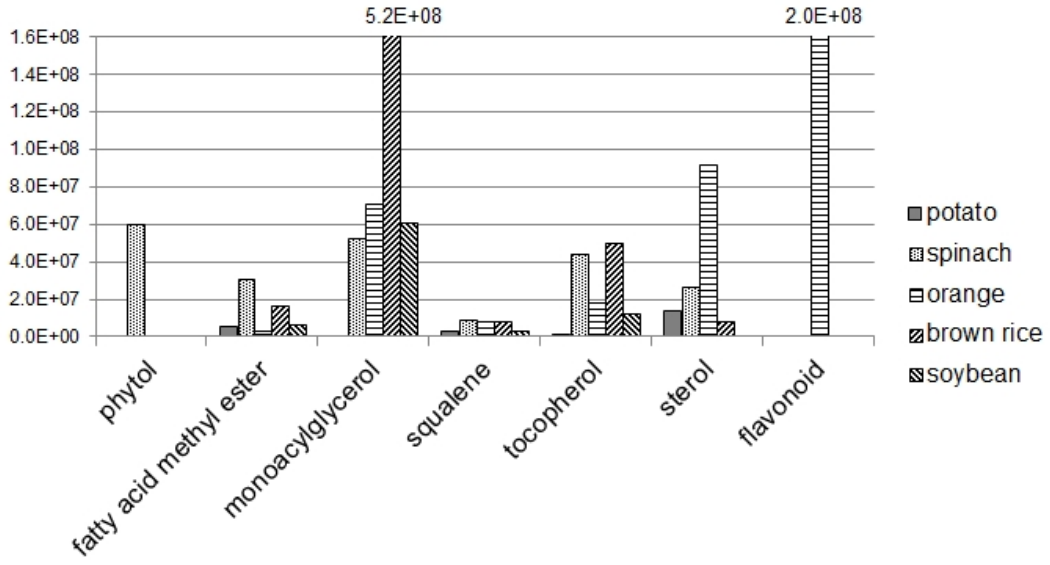


Fig. 3.2 Sum intensity of the total ion chromatogram (TIC) of each component group.

monoacylglycerols, tocopherols, and squalene. In the spinach sample, a fatty acid ester and phytol were also observed. The brown rice sample also contained a fatty acid ester, while the orange sample contained flavonoids. The sum intensity of the total ion chromatogram (TIC) of each component group is shown in Fig. 3.2. Monoacylglycerol, tocopherol, and sterol, which showed high intensity, were chosen for the next experiment. Phytol, which was characteristically contained in the spinach sample because the spinach showed the second-highest matrix effect (from the results of Chapter 2) was also chosen.

3.3.2 Experiment 2: Evaluation of the components which cause the matrix enhancement effect

All results of the matrix effect values are shown in Table 3.1-3.4. The retention time (RT) of phytol was 14.72 min. Triadimenol-2 (RT: 14.71 min) could not be measured because phytol interfered. When the phytol concentration exceeded 100 ppm, the matrix effect values of the pesticides that eluted after phytol were over 120 %. The matrix effect values of the pesticides that eluted 3-4 min earlier than phytol exceeded 120 % in the 1000 ppm solution. However, the matrix effect values of almost all pesticides, except for isophenphos, pyraclofos, and bitertanol, were less than 200 %.

The RT of α-tocopherol was 22.87 min, and it eluted later than deltamethrin (RT: 22.29 min). Although the matrix effect was observed in pesticides that eluted about 3 min earlier than α-tocopherol in solutions over 500 ppm, the matrix effect values were less than 200 %, even in the 1000 ppm solution.

The RT of stigmasterol was 24.46 min, which eluted about 2 min later than deltamethrin. The matrix effect was observed in the pesticides that eluted about 5 min earlier than stigmasterol in solutions over 200 ppm, and in almost all pesticides in the

1000 ppm solution. The matrix effect values of some pesticides, such as carbofuran, pyraclofos, bitertanol, fluquinconazole, fluridone, fenvarelate, difenoconazole, and deltamethrin, exceeded 200%.

The RTs of 4 monoacylglycerols (1-monomyristin, 1-monopalmitin, 1-mono olein, and 1-monostearin) were 16.39 min, 18.06 min, 19.46 min, and 19.62 min, respectively. As of the concentration of monoacylglycerols increased, the matrix effect became higher. The matrix effect values of pesticides that eluted after 1-monoolein in the 10 ppm solution, 1-monomyristin in the 100 ppm solution, and about 4-5 min earlier than 1-monomyristin in the 200 ppm solution exceeded 200 %. In the 500 ppm solution, the matrix effect values of almost all pesticides showed over 200 %, except ethoprophos, simazine, γ-BHC, propyzamide, diazinon, tri-allate, propanil, vinclozolin, alachlor, pirimifos-methyl, chlorpyrifos, and triadimefon. Moreover, the matrix effect values of some pesticides, such as pyraclofos, bitertanol, fluquinconazole, fluridone, and deltamethrin, were over 500 % higher than they were in the 100 ppm solution.

Several components that could cause the matrix effect were tested. The matrix effect was observed in pesticides that eluted after (or from a few minutes before) these components when the matrix solution reached a certain concentration: phytol (100 ppm), α-tocopherol (500 ppm), stigmasterol (200 ppm) and monoacylglycerol (10 ppm). Monoacylglycerol showed the most remarkable matrix effect among them. Five types of monoacylglycerol: 1-monomyristin, 1-monopalmitin, 1-monolinolein, 2-monopalmitin, and 2-monolinolein, were detected in the brown rice sample that showed the highest matrix effect values (from the results of Chapter 2).

Monoacylglycerols are the decomposed or precursor components of fats. Although the fatty acids that are also decomposed or precursor components of fats are contained at percentage order in the agricultural products, they are excluded during sample

cleanup. Monoacylglycerols remained because the chemical property was similar to part of the pesticides. The molecular weight of monoacylglycerols is 302-358, and their log $P_{o/w}$ is 5.1-7.2. On the other hand, the molecular weight of the pesticides in this study is 201-505, and their $P_{o/w}$ is 1.6-6.7. Because monoacylglycerols have two hydroxyl groups, they could adsorb the active site in the GC-MS system. It was also found that the matrix effect in the brown rice sample was similar to that in the 100 ppm monoacylglycerol solution. It was guessed that about 100 ppm of monoacylglycerol was contained in the brown rice sample.

Before these experimental result were determined, it was considered that these components were used as pseudo matrix, such as analyte protectants[24)-26)] if they could not be removed by sample cleanup. However, this was not practical since the matrix effect value changed as the concentration of the matrix solution changed. Moreover, the concentration of these components could be change according to the production area or the season.

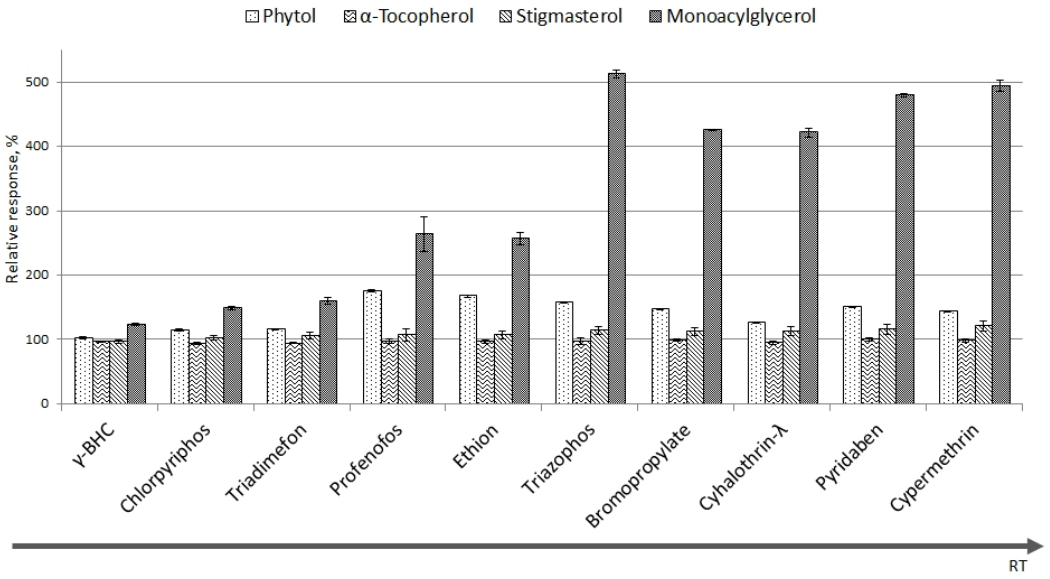
Based on these results, the importance of cleanup to eliminate monoacylglycerols, as well as possibly reducing the matrix effect, was considered. Akiyama *et al.* demonstrated that the gel permeation chromatography (GPC) and the primary-secondary amine (PSA) column were not good enough to remove cholesterol, fatty acid, and glycerin-mono-fatty acid (monoacylglycerols), while adding florisil or silica gel column was effective.[35)] In our other study, we demonstrated that monoacylglycerols were eluted together with pesticides from the PSA and the aminopropylsilanized silica gel (NH_2) column, but a florisil and a silica gel column removed them.[36)]

Sterols were also attributable components to the matrix effect. According to the method by the MHLW, the C18 column cleanup is applied to remove fats from grains,

beans, and seeds. We used the C18 column cleanup for brown rice and soybean, but it was not applied to potato, spinach and orange. Therefore, sterols remained in these sample solutions. However, applying the C18 column cleanup to all samples can effectively eliminate sterols and reduce the matrix effect.

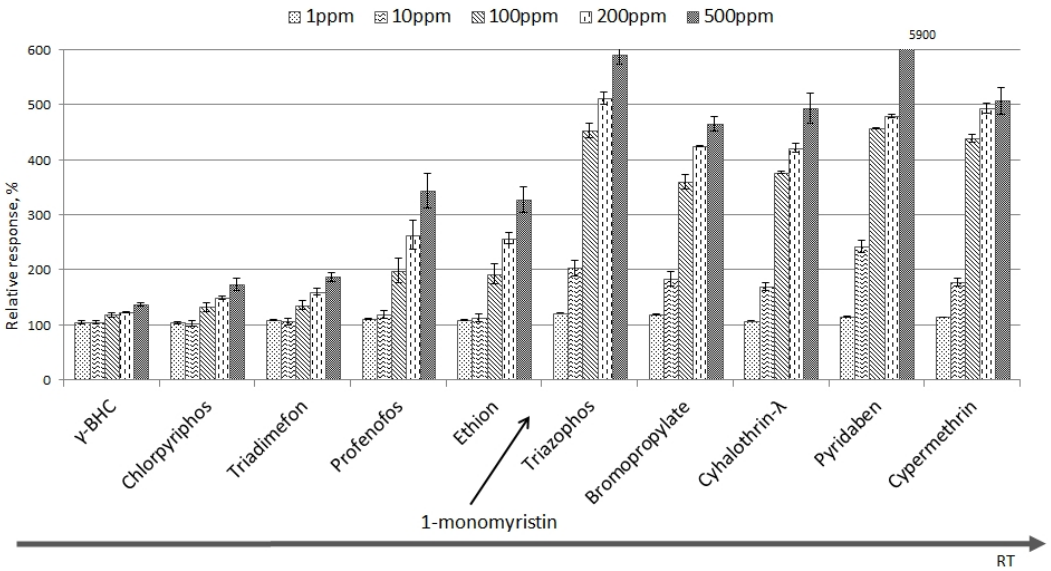
Although only phytol, tocopherol, sterol, and monoacylglycerols were tested, other components, such as fatty acid ester, squalene, and flavonoid need to be evaluated. There is also room to investigate the concentrations in various agricultural products or the samples in which origins (production area, season, etc.) differ. Nevertheless, it is notable that the components that caused the matrix effect were determined.

Fig. 3.3 shows the matrix enhancement effect in each 200 ppm matrix solution (phytol, α-tocopherol, stigmasterol and monoacylglycerol), and Fig. 3.4 shows it in each concentration of monoacylglycerol solution. Fig. 3.5 shows the representative structure of monoacylglycerols and a view showing a frame format of behavior of pesticide and monoacylglycerol in GC-MS.



*Relative response (%) = Relative response of pesticide in each matrix solution to that of the matrix-free standard solution

Fig. 3.3 Matrix enhancement effect in each 200 ppm matrix solution.



*Relative response (%) = Relative response of pesticide in monoacylglycerol solution to that of the matrix-free standard solution

Fig. 3.4 Matrix enhancement effect in each concentration of monoacylglycerol solution.

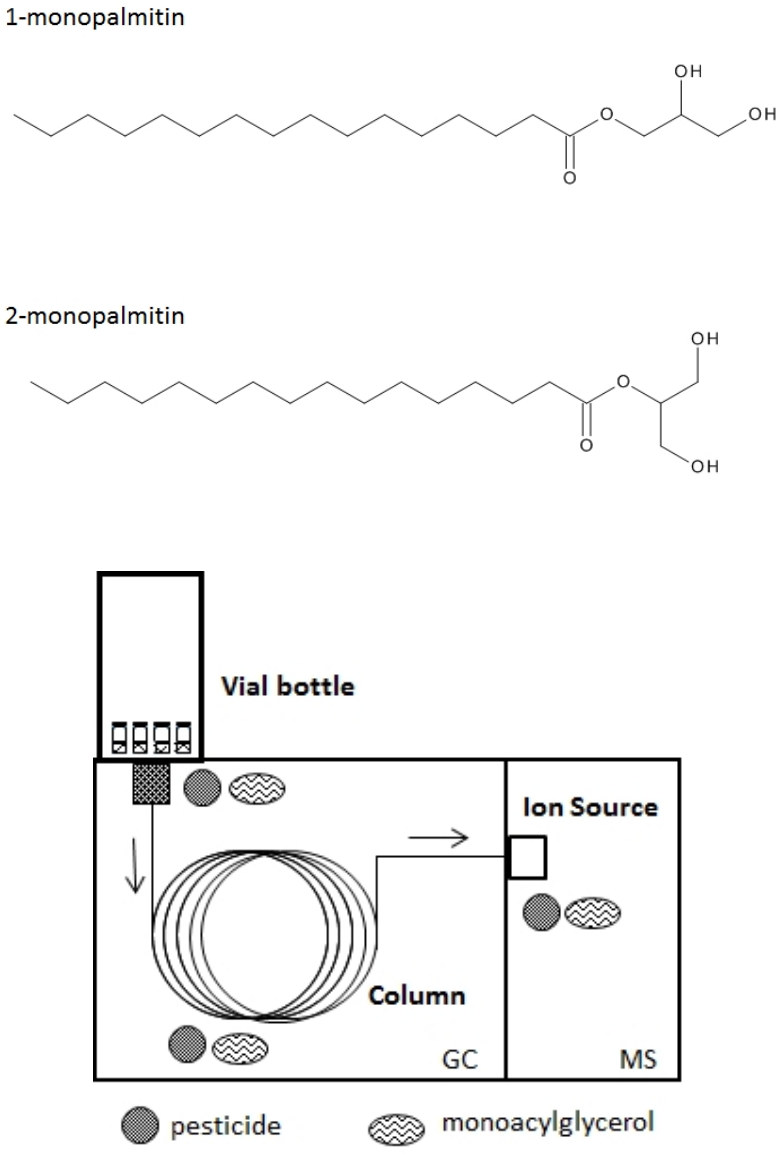


Fig. 3.5 Representitive structures of monoacylglycerols (upper), and behavior of pesticide and monoacylglycerol in GC-MS (lower).

Table 3.1. Matrix effect value of each pesticide in phytol solution

Compound	RT	Monitor ion,	Relative response, % [a)]											
			1ppm		10ppm		100ppm		200ppm		500ppm		1000ppm	
		m/z	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Propoxur	9.70	152	89.1	16	95.6	1.1	101	15	108	1.6	104	6.1	122	6.8
Ethoprophos	10.01	158	92.4	14	100	3.5	104	7	107	4.9	108	4.6	119	6.2
Carbofuran	11.12	164	86.5	17	89.8	1.0	96.9	20	108	2.1	104	4.7	129	9.5
Shimazine	11.16	201	92.9	15	101	4.2	103	13	107	2.5	107	3.4	116	0.6
PCNB	11.41	237	93.8	15	100	2.8	101	9	103	3.1	101	1.7	109	3.5
γ-BHC	11.52	219	93.4	17	97.8	2.8	101	8	103	4.0	100	2.2	106	0.6
Propyzamide	11.58	173	92.9	15	100	5.0	104	12	109	3.2	108	3.3	116	3.1
Diazinon	11.59	304	93.8	14	103	3.2	107	8	112	4.5	112	3.9	124	4.2
Tri-allate	12.02	268	92.1	16	103	3.7	110	9	114	2.2	114	2.1	122	2.4
Propanil	12.54	161	92.7	17	104	4.3	113	16	120	3.2	120	2.9	133	0.7
Vinclozoline	12.68	285	93.9	16	105	4.3	116	13	121	2.8	119	2.3	126	0.3
Alachlor	12.75	160	92.8	16	103	3.2	109	12	113	3.0	113	3.4	122	2.4
Parathion-methyl	12.75	263	91.7	16	103	5.8	107	14	113	3.9	112	4.1	126	8.9
Pirimifos-methyl	13.14	290	92.3	16	105	4.5	120	13	128	2.5	127	3.2	139	2.5
Fenitrothion	13.23	277	90.9	16	103	5.2	112	13	119	4.3	119	5.4	135	3.0
Metolachlor	13.50	162	92.1	15	103	4.5	110	12	118	1.0	118	3.7	129	3.2
Chlorpyriphos	13.53	314	90.9	15	101	5.8	108	12	115	2.4	113	3.6	123	1.8
Parathion	13.69	291	94.5	15	102	5.9	110	18	116	4.1	116	5.0	132	6.9
Triadimefon	13.74	208	88.4	16	100	5.1	108	15	115	2.3	114	3.5	126	2.0
Fipronil	14.19	367	88.8	17	96.7	3.1	106	14	123	4.4	118	7.5	140	5.6
Isophenphos	14.31	213	91.8	16	106	6.4	122	15	130	4.5	136	5.7	204	9.0
CVP-Z	14.35	267	90.7	14	101	5.7	111	15	124	4.7	127	4.6	145	6.3
Triadimenol-1	14.56	168	87.3	15	107	8.9	129	16	155	3.1	182	1.8	114	12
Triadimenol-2[c)]	14.71	168	104	17	-	-	-	-	-	-	-	-	-	-
Phytol [b)]	14.72	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetrachlorvinphos	14.89	329	97.7	19	120	4.9	160	29	177	2.6	171	4.1	191	2.3
Endosulfan-α	15.16	241	93.5	16	107	6.0	128	16	135	4.1	132	0.1	134	2
Flutranil	15.20	173	98.5	18	123	5.9	158	25	177	2.3	174	5.2	197	9.4

continued

Isoprothiolane	15.35	118	95.1	16	113	5.5	144	23	157	0.5	157	0.9	174	3.8
Profenofos	15.41	337	94.3	16	117	3.6	151	25	175	3.2	173	4.8	192	5.1
Oxyfluorfen	15.53	252	95.4	17	110	4.2	141	25	162	1.4	162	3.3	188	11
Myclobutanil	15.56	179	98.1	17	108	7.1	138	23	155	1.8	157	5.8	175	9.3
Buprofezin	15.61	172	95.8	17	111	6.3	139	22	152	3.8	149	2.3	160	1.2
Cyproconazole	15.93	222	95.4	18	115	4.3	139	23	158	3.6	160	1.8	176	3.7
Chlorbenzilate	16.10	251	92.9	16	108	5.3	134	19	154	4.2	158	5.4	173	4.0
Ethion	16.24	231	93.1	17	111	6.0	144	24	167	3.4	169	9.5	189	5.6
Triazophos	16.54	257	92.4	17	108	1.8	136	27	157	0.7	153	7.3	179	4.1
Propiconazole-1	16.84	259	92.4	17	108	4.7	135	24	152	1.1	154	7.0	177	4.7
Propiconazole-2	16.95	259	88.6	17	111	3.6	135	28	148	4.3	146	3.8	167	4.1
Propargite	17.24	150	93.8	17	108	5.2	124	20	141	6.0	131	9.2	149	0.7
Tebuconazole	17.25	250	90.6	15	105	2.6	131	24	151	1.4	158	9.4	182	7.7
Phosmet	17.89	160	92.0	21	104	0.9	130	30	146	1.7	140	5.1	169	7.6
Bromopropylate	17.92	341	90.8	18	106	7.6	128	23	147	2.8	153	8.6	176	3.3
Fenpropathrin	17.99	181	92.1	17	109	7.8	133	22	149	2.0	150	3.3	167	3.4
Methoxychlor	18.01	227	91.6	19	104	5.1	122	24	140	0.5	135	6.3	156	4.1
Cyhalothrin-λ	18.57	181	91.9	15	85.8	4.5	108	20	126	1.6	130	7.9	153	7.4
Pyriproxyfen	18.65	136	94.3	16	91.9	4.8	111	20	123	0.2	122	4.1	136	4.0
Cyhalothrin-γ	18.74	181	65.4	14	106	4.0	130	25	149	2.4	155	9.1	176	10
Pyraclofos	19.29	360	90.3	23	103	2.2	131	36	151	0.4	159	3.4	214	20
Bitertanol	19.56	170	91.5	22	101	1.9	129	32	155	1.8	167	4.6	223	12
Pyridaben	19.79	147	92.8	18	108	3.7	132	23	150	2.2	154	9.6	179	8.8
Fenbuconazole	19.80	340	93.2	20	102	0.1	118	22	131	0.7	128	4.2	149	6.6
Fluquinconazole	20.17	198	96.8	24	103	1.6	120	34	135	3.7	134	11	170	16
Cypermethrin	20.54	181	93.8	21	104	2.3	125	26	144	1.3	146	8.4	177	7.2
Fluridone	21.04	328	89.5	31	101	8.3	134	35	148	1.5	152	4.3	198	17
Fenvarelate-1	21.38	225	90.5	19	104	0.6	124	28	142	1.0	142	10	179	13
Fenvarelate-2	21.61	225	91.5	20	95.0	0.5	117	30	133	5.2	133	9.0	177	9.4
Difenoconazole	22.03	323	88.6	25	102	10	114	33	127	2.4	127	6.7	171	21
Deltamethrin	22.29	181	92.7	24	101	2.3	116	31	132	1.2	132	6.9	169	17

[a)]Relative response of the pesticide in each sample solution to that of the standard solution

[b)]Matrix component, [c)]Phytol interfered triadimenol-2

Table 3.2 Matrix effect value of each pesticide in α-tocopherol solution

Compound	RT	Monitor ion, *m/z*	Relative response, % [a)] 1ppm Mean	1ppm SD	10ppm Mean	10ppm SD	100ppm Mean	100ppm SD	200ppm Mean	200ppm SD	500ppm Mean	500ppm SD	1000ppm Mean	1000ppm SD
Propoxur	9.70	152	102	12	90.9	3.0	93	12	87.2	10.0	106	11	108	16
Ethoprophos	10.01	158	102	7.1	98.5	2.9	104	12	96.7	7.2	114	12	114	14
Carbofuran	11.12	164	106	15	90.0	7.9	88	12	79.9	11.2	97.3	8.5	96.2	18
Shimazine	11.16	201	104	7.4	99.4	1.9	104	11	95.2	6.7	111	11	111	11
PCNB	11.41	237	104	6.2	98.8	1.2	101	7.0	94.5	4.3	109	5.1	108	7.0
γ-BHC	11.52	219	103	7.2	97.9	0.7	101	6.7	96.3	0.6	110	9.2	103	4.7
Propyzamide	11.59	173	103	7.0	99.1	1.3	103	11	96.0	5.5	112	10	112	12
Diazinon	11.60	304	101	2.9	96.6	1.6	100	9.1	94.0	5.4	111	8.2	111	11
Tri-allate	12.02	268	101	4.8	99.3	0.7	102	8.4	94.5	4.7	109	6.6	107	8.7
Propanil	12.54	161	102	11	95.2	1.5	100	14	92.1	5.8	111	11	110	15
Vinclozoline	12.68	285	103	8.5	98.9	0.8	102	10	92.8	4.0	108	7.9	107	8.7
Alachlor	12.76	160	102	7.3	98.0	1.9	102	10	93.2	4.6	109	8.3	109	10
Parathion-methyl	12.76	263	104	9.2	96.9	3.3	102	12	96.2	6.4	118	12	124	14
Pirimifos-methyl	13.14	290	102	7.3	97.6	2.3	103	12	94.8	5.9	113	10	114	12
Fenitrothion	13.23	277	104	9.1	96.0	1.4	101	13	94.3	4.8	116	10	119	13
Metolachlor	13.50	162	100	7.2	95.0	1.8	100	10.5	91.3	4.6	108	9.1	108	11
Chlorpyriphos	13.53	314	102	7.9	98.7	1.4	101	9.1	93.9	3.7	113	8.3	111	9.0
Parathion	13.69	291	103	10	95.5	1.6	103	11	98.4	3.2	122	10	126	15
Triadimefon	13.74	208	103	10	97.1	2.2	102	14	93.9	1.9	111	10	112	12
Fipronil	14.19	367	102	14	94.7	2.0	106	15	100	7.0	124	16	126	19
Isophenphos	14.31	213	104	10	103	2	113	15	107	5.4	132	16	132	16
CVP-Z	14.36	267	98.4	10.7	91.5	2.6	100	14	92.1	6.5	114	14	113	17
Triadimenol-1	14.57	168	101	12	92.5	2.4	101	16	97.9	9.0	121	8.1	124	17
Triadimenol-2	14.71	168	105	10	96.0	1.3	109	16	103	5.3	127	13	131	15
Tetrachlorvinphos	14.89	329	104	14	92.2	2.9	102	15	94.6	6.5	116	13	115	15
Endosulfan-α	15.16	241	101	14	96.9	3.3	102	11	96.7	3.9	116	15	121	17
Flutranil	15.20	173	103	6.9	99.1	2.7	106	10	93.3	3.1	105	4.5	103	8.2
Isoprothiolane	15.34	118	101	12	97.0	1.9	104	12	95.3	4.6	117	5.5	111	11

continued

Profenofos	15.41	337	105	15	95.1	3.8	105	15	97.5	7.0	120	10	123	18
Oxyfluorfen	15.53	252	107	12	95.2	0.9	99	15	94.1	4.5	122	13	126	18
Myclobutanil	15.56	179	105	13	96.6	1.9	108	14	98.1	4.3	122	5.0	117	14
Buprofezin	15.61	172	106	10	98.0	0.2	105	13	95.1	3.4	113	9.1	112	11
Cyproconazole	15.93	222	104	13	96.1	3.5	105	14	97.7	6.2	123	8.6	122	16
Chlorbenzilate	16.11	251	102	10	96.2	0.7	104	13	95.5	2.6	117	11	117	13
Ethion	16.24	231	103	12	95.4	3.1	104	15	96.1	6.0	120	15	121	17
Triazophos	16.54	257	108	17	94.0	2.4	104	16	97.2	9.2	121	14	124	16
Propiconazole-1	16.84	259	103	12	94.7	2.8	102	12	97.2	5.8	121	5.0	117	14
Propiconazole-2	16.96	259	102	10	92.0	1.7	102	11	94.0	7.4	115	7.0	110	13
Propargite	17.25	150	106	16	94.3	3.2	106	16	101	4.2	123	5.6	123	13
Tebuconazole	17.26	250	108	14	99.9	5.3	108	18	106	4.3	131	9.4	135	19
Phosmet	17.89	160	106	17	92.3	1.8	100	14	93.0	7.9	116	15	117	14
Bromopropylate	17.92	341	102	11	97.8	0.2	107	15	98.8	3.5	124	8.7	124	14
Fenpropathrin	18.00	181	103	12	94.5	2.9	104	15	94.3	4.8	117	12	115	13
Methoxychlor	18.01	227	103	14	91.3	2.5	99	11	91.2	3.4	112	9.4	112	10
Cyhalothrin-λ	18.57	181	104	14	92.9	3.7	102	15	94.3	4.9	120	15	121	18
Pyriproxyfen	18.65	136	104	14	94.5	1.8	101	15	92.4	5.2	113	13	112	12
Cyhalothrin-γ	18.74	181	103	14	94.8	3.8	104	16	98.0	5.1	124	14	125	16
Pyraclofos	19.29	360	105	16	86.8	0.6	96.7	15	90.0	10.6	116	15	117	15
Bitertanol	19.57	170	114	19	104	1.4	124	15	120	14	160	17	164	20
Pyridaben	19.79	147	107	12	96.3	3.5	107	17	100	5.7	126	16	126	18
Fenbuconazole	19.80	340	105	16	95.2	3.0	104	14	98.3	8.9	124	9.0	120	14
Fluquinconazole	20.18	198	106	16	93.9	1.0	104	17	99.8	12.1	131	16	132	14
Cypermethrin	20.54	181	106	16	94.6	3.0	106	17	98.4	5.4	128	17	131	17
Fluridone	21.05	328	115	16	99.7	1.2	105	14	109	10	143	27	160	10
Fenvarelate-1	21.38	225	104	16	91.5	3.5	109	18	115	12	172	20	182	12
Fenvarelate-2	21.61	225	107	15	94.4	1.6	113	21	113	8.7	157	18	162	13
Difenoconazole	22.04	323	114	21	101	2.1	124	17	129	13	190	28	192	15
Deltamethrin	22.29	181	109	18	97.0	1.4	114	20	113	10	149	18	151	8.7
α-Tocopherol [b)]	22.87	-	-	-	-	-	-	-	-	-	-	-	-	-

[a)]Relative response of the pesticide in each sample solution to that of the standard solution

[b)]Matrix component

Table 3.3 Matrix effect value of each pesticide in stigmasterol solution

Compound	RT	Monitor ion, *m/z*	Relative response, % [a)]											
			1ppm		10ppm		100ppm		200ppm		500ppm		1000ppm	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Propoxur	9.70	152	99.1	5.5	101	4.3	109	10	125	17	141	12	167	21
Ethoprophos	10.01	158	94.3	4.2	89.6	1.3	98.3	16	100	12	107	7.5	120	15
Carbofuran	11.12	164	101	9.7	119	19	119	6.6	145	25	182	15	225	27
Shimazine	11.16	201	97.9	2.8	102	9.9	97.6	7.5	103	11	107	3.2	119	10
PCNB	11.40	237	98.0	3.4	98.7	6.0	96.8	5.4	101	8.2	105	0.4	116	8.2
γ-BHC	11.52	219	95.9	2.2	98.7	5.7	97.2	2.9	97.0	5.1	98.2	0.1	108	5.4
Propyzamide	11.58	173	98.3	3.9	101	7.8	98.0	7.0	103	12	108	4.4	120	11
Diazinon	11.59	304	94.6	3.1	96.6	6.0	98.4	6.9	98.7	10	105	7.5	119	4.0
Tri-allate	12.02	268	99.5	3.1	97.9	6.9	96.7	5.6	98.9	7.9	102	3.5	111	7.8
Propanil	12.54	161	97.7	3.2	102	11	90.2	6.6	98.6	11	106	3.0	129	20
Vinclozoline	12.68	285	92.0	2.5	99.4	12	93.6	5.9	98.4	5.9	100	2.2	107	6.5
Alachlor	12.75	160	96.9	3.3	100	8.5	97.3	6.9	102	8.8	106	1.9	116	10
Parathion-methyl	12.75	263	96.6	4.3	104	14	100	5.7	111	14	122	3.1	143	16
Pirimifos-methyl	13.13	290	96.8	3.3	100	8.2	96.4	6.8	102	10	107	2.8	120	10
Fenitrothion	13.23	277	97.1	5.5	102	12	97.4	6.4	109	13	119	4.4	134	14
Metolachlor	13.50	162	99.2	5.4	101	9.2	96.8	5.3	102	10	107	3.0	120	11
Chlorpyriphos	13.52	314	100	2.5	104	8.2	98.7	6.8	102	8.5	107	0.0	121	10
Parathion	13.68	291	92.3	7.3	99.2	15	93.5	7.7	109	15	127	5.2	139	14
Triadimefon	13.74	208	98.9	4.0	104	13	94.5	4.9	105	11	111	3.5	124	13
Fipronil	14.19	367	101	5.8	108	12	101	7.1	114	15	124	4.2	146	17
Isophenphos	14.31	213	96.9	4.4	102	10	97.5	8.5	109	14	121	5.5	138	16
CVP-Z	14.36	267	98.7	6.5	105	11	101	9.3	111	14	125	13	142	16
Triadimenol-1	14.57	168	94.7	3.9	102	15	96.6	5.1	110	10	124	0.6	144	17
Triadimenol-2	14.71	168	98.7	3.4	108	12	103	6.5	118	15	131	5.7	156	18
Tetrachlorvinphos	14.89	329	97.8	6.9	104	13	95.8	8.4	115	17	135	6.7	167	19
Endosulfan-α	15.16	241	97.0	5.2	104	12	95.9	7.5	106	13	117	1.8	133	17
Flutranil	15.20	173	97.7	3.2	100	6.0	93.7	3.2	96.9	6.8	100	1.7	107	4.9
Isoprothiolane	15.35	118	96.5	5.0	104	15	95.1	5.3	106	11	113	0.5	127	12

continued

Profenofos	15.41	337	93.8	5.9	96.9	14	91.5	10	108	19	123	7.6	139	17
Oxyfluorfen	15.53	252	96.3	7.6	105	18	97.7	7.7	116	14	132	3.6	157	20
Myclobutanil	15.56	179	98.7	6.6	105	16	96.9	6.1	108	11	114	1.7	131	8.5
Buprofezin	15.61	172	97.6	6.5	103	12	95.4	6.9	102	10	108	1.7	119	8.8
Cyproconazole	15.93	222	101	6.0	107	13	101	5.5	113	13	124	1.1	148	16
Chlorbenzilate	16.10	251	99.7	5.8	106	13	98.6	5.9	109	10	119	2.0	137	14
Ethion	16.23	231	97.6	5.0	103	12	96.1	6.8	106	12	117	5.1	136	17
Triazophos	16.54	257	101	6.8	109	17	97.0	3.5	114	12	129	0.9	156	15
Propiconazole-1	16.85	259	100	6.4	111	14	103	4.5	115	13	126	1.0	148	16
Propiconazole-2	16.95	259	101	3.3	109	18	98.7	10	113	14	121	0.8	138	3.3
Propargite	17.24	150	101	3.5	114	15	109	10	117	19	130	1.3	149	15
Tebuconazole	17.26	250	110	8.3	109	21	102	10	118	16	137	6.0	167	19
Phosmet	17.89	160	99.0	5.8	108	17	97.3	4.7	117	15	140	3.8	176	21
Bromopropylate	17.91	341	96.6	7.1	103	13	96.6	6.1	112	13	129	5.4	152	17
Fenpropathrin	17.99	181	95.9	5.3	103	16	95.9	6.9	105	12	117	2.0	136	14
Methoxychlor	18.01	227	96.7	4.8	105	14	95.5	4.8	105	9.0	114	0.7	130	9.4
Cyhalothrin-λ	18.56	181	96.3	5.1	105	17	98.0	6.1	113	13	130	2.0	155	17
Pyriproxyfen	18.65	136	96.3	5.2	104	16	93.8	5.4	105	10	117	2.6	134	6.7
Cyhalothrin-γ	18.74	181	97.8	4.7	105	18	98.3	5.0	110	15	124	1.9	151	11
Pyraclofos	19.29	360	99.3	3.0	114	22	104	1.9	139	19	186	11	246	36
Bitertanol	19.57	170	105	7.0	130	23	130	7.6	167	22	203	5.8	241	27
Pyridaben	19.79	147	96.6	5.7	107	18	98.3	7.6	115	16	136	6.0	166	21
Fenbuconazole	19.80	340	97.2	9.4	107	22	98.0	1.7	114	10	128	2.6	156	11
Fluquinconazole	20.18	198	107	5.5	114	22	109	2.9	144	20	190	0.7	224	8.5
Cypermethrin	20.53	181	98.1	5.5	109	17	103	3.7	121	16	146	3.0	176	17
Fluridone	21.07	328	102	7.5	117	38	120	13	151	29	218	4.7	267	42
Fenvarelate-1	21.37	225	101	5.0	113	21	109	3.0	132	16	156	1.9	188	18
Fenvarelate-2	21.61	225	103	4.0	117	27	110	7.0	137	23	162	4.8	205	16
Difenoconazole	22.05	323	95.9	4.8	119	27	117	0.1	156	30	197	3.0	264	20
Deltamethrin	22.28	181	100	3.0	119	24	120	4.7	180	67	179	3.4	226	24
Stigmasterol [b]	24.46	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Relative response of the pesticide in each sample solution to that of the standard solution

[b] Matrix component

Table 3.4 Matrix effect value of each pesticide in monoacylglycerol solution

Compound	RT	Monitor ion, *m/z*	Relative response, % [a)]									
			1ppm		10ppm		100ppm		200ppm		500ppm	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Propoxur	9.62	152	114	6.1	118	9.1	171	28	183	17	228	35
Ethoprophos	9.92	158	101	6.4	95.6	10.7	127	21	144	15	198	27
Carbofuran	11.04	164	119	5.8	122	9.1	179	36	198	29	237	48
Shimazine	11.08	201	105	4.7	102	9.9	123	13	128	7.2	148	16
PCNB	11.32	237	104	5.9	104	11	142	26	169	12	213	26
γ-BHC	11.43	219	104	4.8	104	6.1	118	8.1	123	2.0	136	7
Propyzamide	11.50	173	103	4.2	98.2	9.0	123	15	132	11	158	18
Diazinon	11.52	304	101	6.3	101	9.0	128	17	140	5.5	172	19
Tri-allate	11.94	268	102	6.3	99.7	8.6	117	12	127	3.2	153	15
Propanil	12.46	161	105	2.1	101	11	137	21	153	22	181	24
Vinclozoline	12.60	285	103	5.1	101	8.5	119	12	126	5.1	148	18
Alachlor	12.67	160	104	5.0	100	10	128	20	139	12	168	19
Parathion-methyl	12.67	263	110	4.3	111	14	164	33	199	31	269	63
Pirimifos-methyl	13.06	290	103	5.5	98.7	10	126	20	136	10	168	26
Fenitrothion	13.15	277	108	4.0	110	15	164	34	210	30	291	66
Metolachlor	13.42	162	106	4.3	105	13	153	29	186	22	237	33
Chlorpyriphos	13.44	314	104	4.0	103	10	132	17	148	5.4	173	21
Parathion	13.60	291	114	3.2	114	15	174	41	227	39	324	75
Triadimefon	13.66	208	108	0.8	106	12	136	17	160	11	187	19
Fipronil	14.06	367	109	1.8	110	11	161	30	215	39	293	59
Isophenphos	14.23	213	109	4.2	109	13	157	27	183	21	220	33
CVP-Z	14.28	267	106	2.7	107	15	159	30	196	31	261	57
Triadimenol-1	14.48	168	117	2.4	117	14	187	38	257	43	379	61
Triadimenol-2	14.63	168	110	0.3	112	13	180	36	243	36	337	45
Tetrachlorvinphos	14.81	329	114	3.4	118	12	185	31	238	37	301	55
Endosulfan-α	15.07	241	103	6.4	108	10	187	78	521	414	2511	2211
Flutranil	15.12	173	100	1.2	99.1	12	163	39	215	41	265	39
Isoprothiolane	15.26	118	108	2.5	105	12	166	27	192	25	248	41

continued

Profenofos	15.32	337	111	3.0	119	14	198	43	263	54	343	64
Oxyfluorfen	15.45	252	115	0.6	118	13	181	43	250	51	357	87
Myclobutanil	15.48	179	108	0.6	107	7.3	155	26	187	22	215	26
Buprofezin	15.53	172	107	2.5	104	11	140	20	161	16	191	21
Cyproconazole	15.81	222	118	2.5	116	16	191	42	261	45	349	53
Chlorbenzilate	16.02	251	111	1.3	119	15	202	34	254	15	301	27
Ethion	16.15	231	108	2.8	113	15	192	36	257	20	327	47
1-Monomyristin [b)]	16.39	-	-	-	-	-	-	-	-	-	-	-
Triazophos	16.46	257	121	0.6	203	29	453	28	512	22	590	32
Propiconazole-1	16.75	259	115	0.3	175	23	344	26	391	18	399	34
Propiconazole-2	16.87	259	117	4.4	157	13	261	17	287	5.9	319	19
Propargite	17.17	150	110	1.9	141	14	219	6.4	256	3.5	207	5
Tebuconazole	17.17	250	119	0.1	172	22	374	32	434	19	507	55
Phosmet	17.80	160	121	1.6	171	19	371	30	424	23	422	47
Bromopropylate	17.83	341	119	0.8	183	25	359	27	425	1.5	465	25
Fenpropathrin	17.91	181	111	0.7	145	16	254	12	285	1.3	410	40
Methoxychlor	17.92	227	119	1.4	148	16	256	18	283	5.2	293	39
1-Monopalmitin [b)]	18.06	-	-	-	-	-	-	-	-	-	-	-
Cyhalothrin-λ	18.49	181	107	1.2	170	14	377	3.9	422	13	493	53
Pyriproxyfen	18.57	136	113	0.7	171	18	309	11	331	0.3	344	29
Cyhalothrin-γ	18.67	181	115	7.3	163	17	387	15	464	32	684	106
Pyraclofos	19.20	360	135	2.0	287	40	899	69	1057	62	1058	102
1-Monoolein[b)]	19.46	-	-	-	-	-	-	-	-	-	-	-
Bitertanol	19.48	170	146	1.8	358	41	1153	21	1510	6.6	1732	66
1-Monostearin[b)]	19.62	-	-	-	-	-	-	-	-	-	-	-
Pyridaben	19.71	147	114	0.1	242	22	457	0.5	479	5.9	5902	244
Fenbuconazole	19.72	340	119	0.6	184	12	297	1.6	304	1.4	396	246
Fluquinconazole	20.09	198	123	3.3	246	20	579	12	629	6.6	657	27
Cypermethrin	20.36	181	114	1.0	177	14	438	14	494	18	506	47
Fluridone	20.96	328	122	14	157	11	599	0.6	631	82	713	67
Fenvarelate-1	21.29	225	125	4.6	180	2.7	542	19	662	27	744	149
Fenvarelate-2	21.51	225	114	1.4	169	14	392	9.2	470	28	553	61
Difenoconazole	21.86	323	118	2.4	155	9.0	210	6.6	211	23	221	13

continued

Deltamethrin	22.18	181	131	4.0	180	16	722	57	757	85	864	87

[a)]Relative response of the pesticide in each sample solution to that of the standard solution

[b)]Matrix component

Chapter 4

Matrix Behavior during Sample Preparation Using Metabolomics Analysis Approach for Pesticide Residue Analysis by GC-MS in Agricultural Products

4.1 Introduction

An analytical method for pesticide residues in foods comprising sampling, extraction, and column cleanup steps has been established over the years.[37] Acetone, acetonitrile, or methanol has been used as the extraction solvent because these solvents are easily miscible with the agricultural products and penetrate the tissue of the samples. Liquid/liquid extraction (e.g., *n*-hexane, ethyl acetate, methylene chloride/water) is used to extract the pesticides and remove the polar matrices such as sugars. n-Hexane/acetonitrile extraction is used to remove lipids. Many types of columns can be selected on the basis of the characteristics of the measured compounds and sample matrices. Florisil and silica gel columns have been widely used in the individual method by the MHLW[38] to remove polar matrices. Sometimes, other characteristic columns, such as a silver nitrate containing column to remove sulfur compounds from onion or garlic, have been used.[37] Since around 2000, GC-MS has been widely used, because it provides the simultaneous determination and confirmation of a large number of pesticides instead of using different types of GC detectors. At the same time, the miniaturization of the sample preparation using a minicolumn proceeded. Cairn *et al*. reported that a C18 column, anion-exchange column, and NH_2 column removed the majority of hydrocarbon-like molecules, colored compounds and flavors, and sugars, respectively.[39] Fillion *et al*. also applied C18 and NH_2 columns for 251 pesticides.[40] Akiyama *et al*. demonstrated that a PSA and NH_2 column removed fatty acid and chlorophyll. They adopted the PSA column instead of the NH_2 column because the

recovery rates of some pesticides, having an acid−amide bond in their structure, were low.[41] Ueno *et al*. used gel permeation chromatography (GPC) before the solid phase extraction (SPE) cleanup because GPC separated the compounds by molecular weight.[42] Recently, the QuEChERS method that was developed by Anastassiades *et al*. has been widely used. They reported that dispersive-SPE with PSA was used to remove organic acids, polar pigments, and sugars,[43] and this method was validated for 229 pesticides by Lehotay *et al*.[44] Okihashi *et al*. developed a modified QuEChERS method and used the SPE column cleanup instead of the dispersive-SPE.[45] In all of the studies, the important things were how the multiresidue analysis was performed, how the limit of detection (LOD) and the limit of quantitation (LOQ) were reduced, and how the sample preparation was simplified. Although they mentioned which matrices were removed by which cleanup, they did not indicate the specific component's name. They saw the disappearance of the color using graphite carbon black (GCB) or a decrease in the peak of the fatty acid as a chromatographic interference. They might also estimate the removal matrices theoretically on the basis of their physical and chemical properties. In the multiresidue analysis of pesticides, large matrix interference on the chromatographic peaks and matrix enhancement effect[3)-12)] are problems. The matrix enhancement effect means that the response of pesticides in foods is higher than that in the matrix-free standard solution. These phenomena cause difficulty in the accurate quantification of the pesticides. Although GC-MS/MS has been widely used for its selectivity in the past few years, the matrix enhancement effect remains.[13),46)] The matrix effect is caused by the adsorption of the pesticides on the active sites in the injection port,[3),7)] column, and ion source.[47)] Meanwhile, a metabolomics analysis is the metabolic profiling of metabolites from polar compounds, such as sugars, organic acids, and amino acids, to mid-low polar compounds, such as fatty acids and sterols in cells of

all organisms (e.g., plants, humans, microbes). For all of these compounds, GC-MS is one of the most popular techniques because GC-MS is a robust and highly sensitive method with many databases.[33),48)-51)] The peak detection software, such as the Automated Mass Spectral Deconvolution and Identification System (AMDIS) by the NIST, accelerated the multitarget metabolic profiling analysis using GC-MS for complex biological samples. Fiehn *et al*. identified the metabolites in Arabidopsis using GC-MS and AMDIS.[48)] The general metabolic profiling analysis procedure[32)] is as follows: (1) The samples are extracted with a mixture of methanol, water, and chloroform or with a mixture of acetonitrile, water, and isopropanol, etc. (2) The dried samples are methoxyaminated, followed by trimethylsilylation. (3) GC-MS analysis is performed followed by (4) data analysis. If the detailed matrices, which interfere with the pesticide chromatographic peaks or which cause the matrix effect, are determined, we can efficiently remove them. In the present study, we focused on a metabolomics analysis approach to identify the matrix components in each step of the sample preparation. The first study was the matrix profiling of sample solutions extracted by two different popular solvents, acetone and acetonitrile, using three different types of agricultural products, that is, spinach, orange, and brown rice. These samples are representative agricultural products based on the “the validation guideline for pesticide residue analysis in foods” by the MHLW, Japan.[52)] Acetone is used in the method[53)] by the U.S. Food and Drug Administration (FDA) and in the Notification method by the MHLW.2 These methods are based on the reports by Luke et al.[54),55)] On the other hand, acetonitrile was adopted by the California Department of Food and Agriculture (CFDA)[53)] and has been used in the PLS for multiresidue analysis.[2)] These methods are based on the report by Fillion et al.[39),56)] According to The Merck Index,[57)] acetone is a solvent for extracting fats, oils, waxes, resins, rubber, plastics, etc. Acetonitrile is

miscible with water, methanol, methyl acetate, ethyl acetate, and acetone but immiscible in many saturated hydrocarbons. This means that acetone easily dissolves many polar to nonpolar compounds, whereas acetonitrile dissolves most compounds except for the nonpolar compounds.

The next experiment was the column cleanup efficiency using different types of SPE cartridge columns, that is, Florisil, silica gel, NH_2, PSA, and GCB columns, which are commonly used for pesticide residue analyses. The brown rice extract by acetone was selected in this experiment. The purpose of this study was to investigate whether the metabolomics analysis approach could be applied to identify the matrix components and to know the matrix behavior during the sample preparation using common solvents and SPE columns.

4.2 Experimental

4.2.1 Reagents and Apparatus

Acetone and acetonitrile, high purity grades for pesticide residue analysis, were obtained from Wako Pure Chemical Industries (Osaka, Japan). Methoxyamine hydrochloride and pyridine were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) with 1% trimethylchlorosilan (TMCS) was obtained from Thermo Fisher Scientific (Rockford, IL, USA).

The sep-pak plus silica gel column (filler weight: 690 mg), florisil column (910 mg) and NH_2 column (360 mg) were obtained from Waters (Milford, MA, USA). The Bond elute C18 column (filler weight/column size: 1 g/6 mL), PSA column (500 mg/6 mL) and GCB column (500 mg/6 mL) were obtained from Agilent Technologies (Lake Forest, CA, USA). The characteristics of each SPE are listed in Table 4.1.

Table 4.1. **Characteristics of each SPE.**

name	filler	filler weight (mg)	reaction	common elute solvent
C18	octadecyl	1,000	Reverse phase and distribution	acetonitrile
Silica gel	silica gel	690	Normal phase and adsorption	acetone/*n*-hexane, ethylacetate, etc
Florisil	magnesium silicate	910	Normal phase and adsorption	acetone/*n*-hexane, ethylacetate, etc
NH_2	aminopropylsilanized silicagel	360	Ion exchange, normal phase and distribution	acetone, ethylacetate, etc
PSA	ethylenesiamine-*N*-propylsilanized silica gel	500	Ion exchange, normal phase and distribution	acetone, ethylacetate, etc
GCB	graphite carbon black	500	Adsorption	acetone, etc

The spinach and orange were purchased from local food stores in Japan. The brown rice was harvested in Ibaraki prefecture, Japan.

4.2.2 Sample Preparation for Experiment 1.

Acetone and acetonitrile were selected as the representative extraction solvents for the pesticide residue analysis. The sample solution after extraction by acetone is usually evaporated to dryness. On the other hand, the sample solution after being extracted by acetonitrile is easily separated from the water layer by adding sodium chloride.[2)] In this experiment, because the purpose was to compare the difference between the two extractive solvents, the same analytical method was performed, and the C18 cartridge column was used instead of liquid-liquid extraction.[14),58)] Twenty gram (wet weight) aliquots of homogenized spinach and orange were extracted with 100 mL of acetone or acetonitrile using a homogenizer for 3 min. A 10 g (dry weight) sample of homogenized brown rice was extracted with 100 mL of acetone and acetonitrile after soaking in 20 mL of water for 15 min. For the dried samples, the samples are soaked in water to efficiently extract the pesticides.[2)] The mixture was then filtered by vacuum suction. The residual cake was washed with 50 mL of the solvent and filtered. The filtrates were combined and concentrated by a rotary-evaporator in a water bath below 40 °C. After a C18 cartridge column had been conditioned with 5 mL of acetonitrile and 5 mL of water, the concentrated sample solution (adjusted to 20 g by adding water) was loaded on the column. The inside of the flask was washed with 5 mL of water/acetonitrile (80:20, v/v) and this rinse was also passed through the column, and then discarded. The column was vacuum-dried for one min. A 10 mL aliquot of acetonitrile was passed through and collected. The eluate was evaporated to dryness under 40 °C and the residue was dissolved in 1 mL of acetone. These sample solutions were then used for the next step,

derivatization.

4.2.3 Sample Preparation for Experiment 2.

A 25 g aliquot of homogenized brown rice was extracted with 250 mL of acetone after soaking in 50 mL of water for 15 min. The mixture then was filtered by vacuum suction. The residue was extracted again with 50 mL of acetone. The residual cake was washed with 100 mL of acetone and filtered. Acetone was added to the filtrates to make a 500 mL solution, and then 80 mL (equivalent to a 4 g sample) was measured for the column cleanup experiment. Each sample solution was concentrated by rotary evaporation in a water bath below 40 °C. The procedure for the C18 column was the same as for experiment 1. After passing through the C18 column, the eluate was evaporated to dryness under 40 °C and the residue was applied to a cleanup test using each SPE. The residue, which was passed only through the C18 cartridge column, was the control sample. The samples treated with each column cleanup were compared to the control samples.

After the silica gel and Florisil columns were rinsed with 5 mL of n-hexane, the residues were loaded. SPE was done as follows: fraction-1 (Fr-1), *n*-hexane 5 mL; Fr-2, acetone/*n*-hexane (5:95, v/v); Fr-3, acetone/*n*-hexane (15:85, v/v); Fr-4, acetone/*n*-hexane (50:50, v/v). As for the NH_2 and PSA columns, the columns were rinsed with 5 mL of acetone, and then the residues were applied and eluted with 5 mL of acetone. As for the GCB column, the column was rinsed with 10 mL of acetonitrile/toluene (75:25, v/v), then the residue was applied and eluted with 10 mL of acetonitrile/toluene (75:25, v/v). All of the eluates were evaporated to dryness under 40 °C, and then the residues were dissolved in 200 μL of acetone. These sample solutions were then followed by derivatization.

To calculate the cleanup efficiency, the samples (n=2) treated by each column were compared to the control samples (passed only through the C18 column). The formula for the elution rate from each column is as follows:

Elution Rate (%) = (Mean value of the intensity of eluted matrix component from each column/Mean value of the intensity of the components passed through the C18 column) × 100

4.2.4 Derivatization

All samples were analyzed by GC-MS with derivatization. The derivatization procedure was applied using a metabolomics technique.[32),33)] Methoxyamination was performed prior to the trimethylsilylation, and this was done to protect the carbonyl groups because α-keto acids tend to undergo chemical loss of carboxyl groups as carbon dioxide if the keto group is left unprotected. The hydrophilic functional groups (e.g., carboxyl, hydroxyl, amino, immino, or sulfuryl groups) are trimethylsililated to remove the hydrogen bond formations to increase the volatility. This also reduces any interaction with the column phase that can cause tailing peaks, a poor sensitivity, and poor chromatographic separation. For derivatization, 100 µL of the sample solution was dried by a centrifugal concentrator. The residue was then methoxyaminated using 10 µL of methoxyamine hydrochloride in pyridine (40 mg/mL) and stored at 30 °C for 90 minutes. For the trimethylsilylation, after the addition of 90 µL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane (MSTFA + 1% TMCS) to the methoxyaminated samples, the mixture was stored at 37 °C for 30 min.

4.2.5 GC-MS Conditions.

A GC-MS analysis was performed using an Agilent 7890A GC system coupled to an Agilent 5975C TAD mass spectrometer (Little Falls, DE, USA). The sample solutions were injected with 1 μL in the split mode (split ratio was set at 10:1) by an Agilent 7693 autoinjector. The inlet temperature was 250 °C. An Agilent fused silica capillary column, DB-5ms DG (5% phenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, with a 10 m nonpolar deactivated precolumn directly connected to DB-5ms) (Folsom, CA, USA) was used. After the sample injection, the oven temperature was maintained at 60°C for 1 min and then ramped to 325 °C at 10 °C /min. The quadrupole was operated in the scan mode (*m/z* 50-600). The transfer line, ion source, and quadrupole were set at 290 °C, 250 °C and 150 °C, respectively. Myristic acid-d_{27} was locked at 16.752 min in to use the Fiehn metabolomics library (Agilent Technologies, Inc.).[32)]

4.2.6 Identification.

All data were deconvoluted by AMDIS and automatically identified the deconvoluted spectra by Fiehn metabolomics library.[32)] The Fiehn metabolomics library was created by Professor Oliver Fiehn[59)], and includes around 1000 metabolites with both mass spectra (derivatized by methoxyamination and trimethylsililation) and "RI Calibration Data". The "RI Calibration Data" is the calibration file between the RI of the fatty acid methyl ester (FAME) and retention time (RT). If the matching scores of the target compounds were low or there were identified compounds, a library search was done by a NIST search directly from AMDIS for more identification. W9N08 (combined library Wiley9 and NIST08, Agilent Technologies, Inc.) and the free database from the Max Planck Institute of Molecular Plant Physiology[60)] were added to

the NIST search program. Although Wiley9 and NIST08 contain many derivatized compounds, the registered name is the trimetylsililated name (e.g., hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester). We drew the structure without derivatization and confirmed it again by its formula in the NIST program. The "Chemistry of Organic Natural Resources" was also used for more confirmation.[61)]

4.3 Results and Discussion

4.3.1 Experiment 1: Difference between two Extractive Solvents, Acetone and Acetonitrile.

Highly concentrated compounds, such as fatty acids, flavonoids, sterols, and terpenoids, were found in the samples. The mean semi-quantified values of five replicates (n = 5) were calculated by the area of the myristic acid-d_{27}. All results are shown in Tables 4.2-4.4.

Some fatty acids, such as palmitic acid and linoleic acid, were extracted at several hundred milligrams per kilogram from all samples. According to the standard tables of food composition in Japan,[62)] palmitic acid, oleic acid, and linoleic acid are contained at 5200, 8000, and 8600 mg/kg, respectively, in the case of brown rice. Although around 90% of the fatty acids were removed by solvent extraction and a C18 column, excess fatty acids still remained at high concentrations in the extracted solutions.

Although pesticides have a wide variety of properties and cannot be completely explained by their *n*-octanol/water partition coefficient (log $P_{O/W}$) value, the log $P_{O/W}$ value is sometimes used to help characterize the pesticides.[58),63)] Because the matrix components have a wide range of properties as well as pesticides, the polarity of the components, the log $P_{O/W}$ value, was used as one of the indicators. The relationship between the matrices and the log $P_{O/W}$ value is shown in Figure 4.4. The matrix

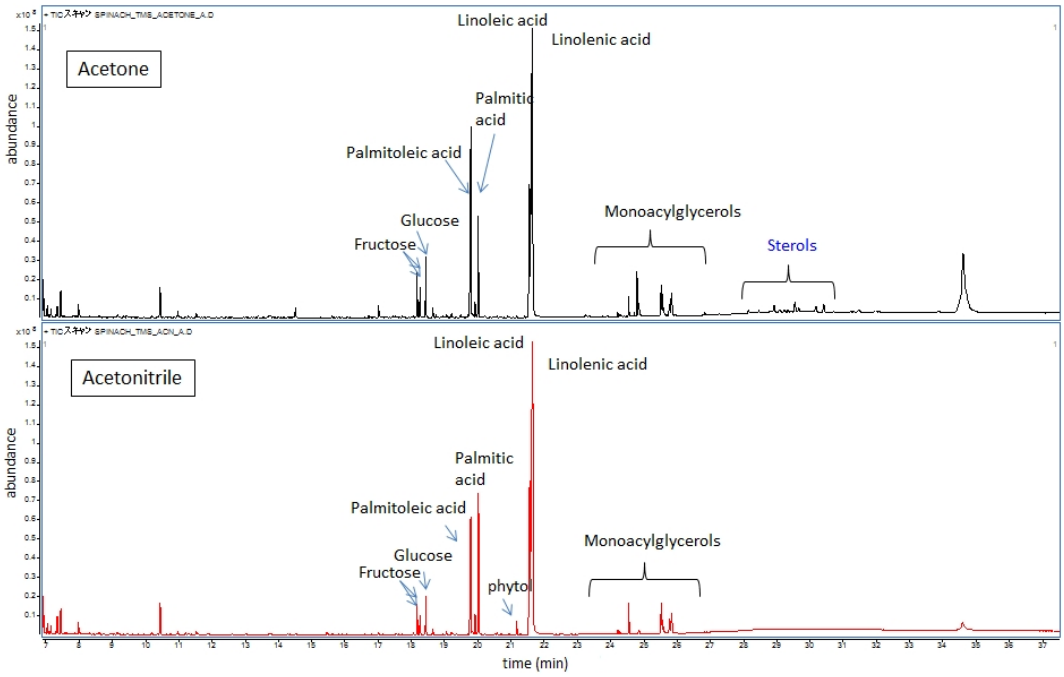


Fig. 4.1 Comparative chromatogram of spinach extraction.

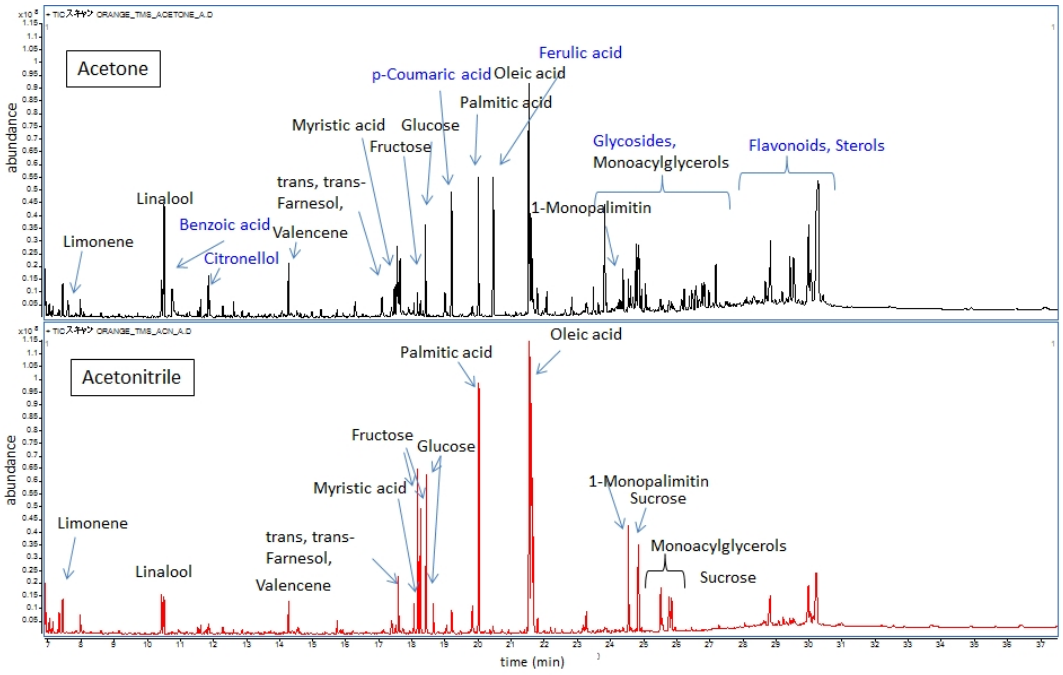


Fig. 4.2 Comparative chromatogram of orange extraction.

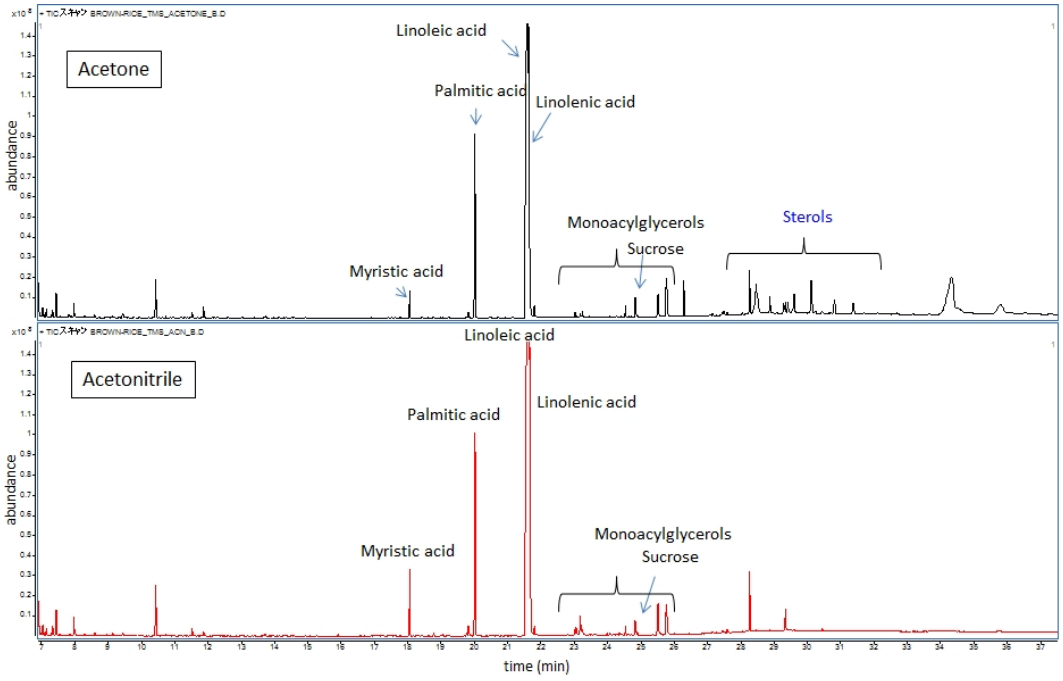


Fig. 4.3 Comparative chromatogram of brown rice extraction.

Table 4.2 Matrix Components in Spinach Extraction

compound name	formula	CAS No.	log $P_{O/W}$	group	concentration	
					Acetone	Acetnitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
myristic acid	$C_{14}H_{28}O_2$	544-63-8	5.79	fatty acid	B	B
pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	6.30	fatty acid	B	B
palmitoleic acid	$C_{16}H_{30}O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	A	A
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.96	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
stearic acid	$C_{18}H_{36}O_2$	57-11-4	8.22	fatty acid	C	C
palmiteladic acid	$C_{16}H_{30}O_2$	-	-	fatty acid	B	B
11-eicosenoic acid	$C20H_{38}O_2$	2462-94-4	8.44	fatty acid	C	C
Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	301-00-8	6.96	fatty acid, ester	C	C
myristin, 1-mono-	$C_{17}H_{30}O4$	589-68-4	5.05	monoacylglycerol	C	C
linolenin, 1-mono-	$C_{21}H_{36}O_4$	18465-99-1	5.41	monoacylglycerol	B	B
palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	B	B
palmitin, 1-mono-	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	A	A
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	A	A
olein, 2-mono-	$C_{21}H_{40}O_4$	3443-84-3	6.94	monoacylglycerol	A	A
stearin, 2-mono-	$C_{21}H_{42}O_4$	621-61-4	7.46	monoacylglycerol	B	B
4-vinylguaiacol	$C_9H_{10}O_2$	7786-61-0	1.93	terpenoid	B	C
phytol	$C_{12}H_{40}O$	150-86-7	8.23	terpenoid	B	B
β-tocopherol	$C_{28}H_{48}O_2$	148-03-8	10.72	tocopherol	<1	<1
α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	B	ND
stigmasterol	$C_{29}H_{48}O$	83-48-7	10.07	sterol	C	C
glucose	$C_6H_{12}O_6$	50-99-7	-2.49	sugar	B	B
fructose	$C_6H_{12}O_6$	57-48-7	-1.47	sugar	B	B
coumaran	C8H8O	496-16-2	2.14	-	C	<1

[a] A: ≧1000 mg/kg, B: ≧10 mg/kg, < 1000 mg/kg, C: ≧1 mg/kg, < 10 mg/kg, <1: <1 mg/kg

Table 4.3 Matrix Components in Orange Extraction

compound name	formula	CAS No.	log $P_{O/W}$	group	concentration	
					Acetone	Acetnitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
myristic acid	$C_{16}H_{32}O_2$	544-63-8	5.79	fatty acid	B	B
pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	6.30	fatty acid	C	C
palmitoleic acid	$C_{16}H_{30}O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	A	A
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.81	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
oleic acid	$C_{18}H_{34}O_2$	112-80-1	7.42	fatty acid	A	A
decanal	$C_{10}H_{20}O$	112-31-2	4.09	aliphatic aldehyde	C	C
dodecanal	$C_{12}H_{24}O$	112-54-9	5.16	aliphatic aldehyde	C	C
p-coumaric acid	$C_9H_8O_3$	7400-08-0	1.01	aromatic carboxylic	A	B
benzoic acid	$C_7H_6O_2$	65-85-0	1.56	aromatic carboxylic	B	C
4-vinylguaiacol	$C_9H_{10}O_2$	7786-61-0	1.93	aromatic carboxylic	B	C
3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	39151-19-4	1.9	aromatic ether, ester	C	C
4-((1E)-3-Hydroxy-1-propenyl)-2-met	$C_{10}H_{12}O_3$		0.92	aromatic alcohol,	B	C
trans-ferulic acid	$C_{10}H_{10}O_4$	537-98-4	0.96	aromatic alcohol,	A	<1
(-)-cis-carane	$C_{10}H_{18}$	2778-68-9	4.77	hydrocarbon	<1	C
3,3',4',5,5',7,8-Heptamethoxyflavone	$C_{22}H_{24}O_9$	-	1.35	flavonoid	A	A
Flavone, 3,3',4,5,5',7-hexamethoxy-	$C_{21}H_{22}O_8$	14813-27-5	2.49	flavonoid	A	A
4H-1-Benzopyran-4-one,	$C_{20}H_{20}O_8$	479-90-3	2.55	flavonoid	B	B
Hesperetin	$C_{16}H_{14}O_6$	520-33-2	2.90	flavonoid	B	B
4H-1-Benzopyran-4-one,	$C_{20}H_{20}O_7$	2306-27-6	3.08	flavonoid	B	C
Naringenin	$C_{15}H_{12}O_5$	480-41-1	3.19	flavonoid	B	<1
flavone, 4',5,6,7-tetramethoxy-	$C_{19}H_{18}O_6$	1168-42-9	3.26	flavonoid	C	<1
5,5'-dimethoxy-3,3'-dimethyl-2,2'-bin	$C_{24}H_{18}O_6$	54215-49-5	4.70	flavonoid	B	<1
4H-1-Benzopyran-4-one,	$C_{20}H_{20}O_7$	2306-27-6		flavonoid	B	C
myristin, 1-mono-	$C_{17}H_{30}O4$	589-68-4	5.05	monoacylglycerol	C	C
linolenin, 1-mono-	$C_{21}H_{36}O_4$	18465-99-1	5.41	monoacylglycerol	C	C

continued

palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	C	C
palmitin, 1-mono-	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	B	B
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	B	B
olein, 2-mono-	$C_{21}H_{40}O_4$	3443-84-3	6.94	monoacylglycerol	B	B
limonin	$C_{26}H_{30}O_8$	1180-71-8	1.66	terpenoid	B	B
2-cyclohexen-1-one,	$C_{10}H_{14}O$	16750-82-6	2.15	terpenoid	C	<1
limonene oxide, trans-	$C_{10}H_{16}O$	6909-30-4	2.43	terpenoid	C	C
4-terpinenol	$C_{10}H_{18}O$	562-74-3	2.54	terpenoid	C	<1
p-mentha-1(7),8(10)-dien-9-ol	$C_{10}H_{16}O$	29548-13-8	2.65	terpenoid	C	C
perilla aldehyde	$C_{10}H_{14}O$	2111-75-3	2.68	terpenoid	C	C
α-terpineol	$C_{10}H_{18}O$	98-55-5	2.79	terpenoid	B	C
bicyclo[4.4.0]dec-2-ene-4-ol,	$C_{15}H_{24}O_2$	-	2.88	terpenoid	C	C
α-citral	$C_{10}H_{16}O$	141-27-5	3.17	terpenoid	B	B
linalool	$C_{10}H_{18}O$	78-70-6	3.28	terpenoid	B	B
citronellol	$C_{10}H_{20}O$	26489-01-0	3.38	terpenoid	B	C
(R)-(+)-citronellal	$C_{10}H_{18}O$	2385-77-5	3.48	terpenoid	C	B
3,4-2H-coumarin,	$C_{14}H_{18}O_2$	-	3.74	terpenoid	C	C
nootkatone	$C_{15}H_{22}O$	4674-50-4	3.84	terpenoid	B	B
2-hexenoic acid, butyl ester, (E)-	$C_{10}H_{18}O_2$	54411-16-4	3.97	terpenoid	B	C
nerol acetate	$C_{12}H_{20}O_2$	141-12-8	4.1	terpenoid	C	C
geraniol acetate	$C_{12}H_{20}O_2$	105-87-3	4.1	terpenoid	C	C
limonene	$C_{10}H_{16}$	138-86-3	4.45	terpenoid	B	B
β-eudesmol	$C_{15}H_{26}O$	473-15-4	4.68	terpenoid	B	B
α-sinenasal	$C_{15}H_{22}O$	4955-32-2	4.86	terpenoid	C	C
farnesol	$C_{15}H_{26}O$	4602-84-0	5.31	terpenoid	B	B
trans, trans-farnesol	$C_{15}H_{26}O$	106-28-5	5.31	terpenoid	B	B
farnesol, acetate	$C_{17}H_{28}O_2$	-	6.14	terpenoid	C	C
(-)-α-panasinsen	$C_{15}H_{24}$	56633-28-4	6.36	terpenoid	C	C
valencene	$C_{15}H_{24}$	4630-07-3	6.49	terpenoid	B	A
β-elemene, (-)-	$C_{15}H_{24}$	110823-68-2	6.63	terpenoid	C	B
caryophyllene	$C_{15}H_{24}$	87-44-5	6.78	terpenoid	C	C
δ-cadinene, (+)-	$C_{15}H_{24}$	483-76-1	6.83	terpenoid	C	B

continued

eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	473-04-1	-	terpenoid	C	C
β-cuvebene	$C_{15}H_{24}$	13744-15-5		terpenoid	C	C
β-tocopherol	$C_{28}H_{48}O_2$	148-03-8	10.72	tocopherol	C	C
α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	C	C
sucrose	$C_{12}H_{22}O_{11}$	57-50-1	-4.49	sugar	B	B
glucose	$C_6H_{12}O_6$	50-99-7	-2.49	sugar	B	B
fructose	$C_6H_{12}O_6$	57-48-7	-1.47	sugar	B	B
4,10-(methanoxymethano)-10H-cyclo	$C_{22}H_{30}O_4$	56786-53-9	-1.47	-	B	C
obacunone	$C_{26}H_{30}O_7$	751-03-1	2.91	-	C	<1
hedycaryol	$C_{15}H_{26}O$	21657-90-9	5.20	-	C	C
chlorpyrifos	**$C_9H_{11}Cl_3NO_3PS$**	**2921-88-2**	**4.77**	**pesticide**	**C**	**C**

[a] A: ≧1000 mg/kg, B: ≧10 mg/kg, < 1000 mg/kg, C: ≧1 mg/kg, < 10 mg/kg, <1: <1 mg/kg

Table 3.4 Matrix Components in Brown Rice Extraction

compound name	formula	CAS No.	log $P_{O/W}$	group	concentration	
					Acetone	Acetnitrile
lauric acid	$C_{12}H_{24}O_2$	143-07-7	4.77	fatty acid	C	C
miristic acid	$C_{16}H_{32}O_2$	57-10-3	5.79	fatty acid	A	A
pentadecanoic acid	$C_{15}H_{34}O_2$	1002-84-2	6.30	fatty acid	C	C
palmitoleic acid	$C_{16}H30O_2$	2091-29-4	6.40	fatty acid	B	B
linolenic acid	$C_{18}H_{30}O_2$	463-40-1	6.52	fatty acid	B	B
palmitic acid	$C_{16}H_{32}O_2$	57-10-3	6.81	fatty acid	A	A
linoleic acid	$C_{18}H_{32}O_2$	60-33-3	7.02	fatty acid	A	A
heptadecenoic acid	$C_{17}H_{32}O_2$	26265-99-6	7.28	fatty acid	C	C
heptadecanoic acid	$C_{17}H_{34}O_2$	506-12-7	7.32	fatty acid	C	C
oleic acid	$C_{18}H4_2O_2$	112-80-1	7.70	fatty acid	A	A
stearic acid	$C_{18}H_{36}O_2$	57-11-4	8.22	fatty acid	B	B
palmitelaidic acid	$C_{16}H_{30}O_2$	-	8.22	fatty acid	C	C
11-eicosenoic acid	$C_{20}H_{38}O_2$	2462-94-4	8.44	fatty acid	C	C
arachidic acid	$C_{20}H_{40}O_2$	506-30-9	8.85	fatty acid	C	C
behenic acid	$C_{22}H_{44}O_2$	112-85-6	9.87	fatty acid	<1	<1
lignoceric acid	$C_{24}H_{48}O_2$	557-59-5	10.89	fatty acid	<1	<1
13-docosenamide, (Z)-	$C_{22}H_{43}NO$	112-84-5	8.87	aliphatic amide	B	<1
butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$		9.24	fatty acid, ester	B	<1
myristin, 1-mono-	$C_{17}H_{30}O4$	589-68-4	5.05	monoacylglycerol	B	B
myristin, 2-mono-	$C_{17}H_{34}O_4$	3443-83-2	5.33	monoacylglycerol	B	B
palmitin, 2-mono-	$C_{19}H_{38}O_4$	23470-00-0	6.14	monoacylglycerol	C	C
palmitin, 1-mono	$C_{19}H_{38}O_4$	542-44-9	6.17	monoacylglycerol	B	B
linolein, 1-mono-	$C_{21}H_{38}O_4$	2277-28-3	6.19	monoacylglycerol	B	B
linolein, 2-mono-	$C_{21}H_{38}O_4$	3443-82-1	6.42	monoacylglycerol	B	B
Stigmasterol	$C_{29}H_{48}O$	83-48-7	10.07	sterol	C	<1
Campesterol	$C_{28}H_{48}O$	474-62-4	10.20	sterol	B	<1
9,19-Cyclolanost-24-en-3-ol,	$C_{30}H_{50}O$	469-38-5	10.31	sterol	B	C
9,19-Cyclolanostan-3-ol,	$C_{31}H_{52}O$	1449-09-8	10.66	sterol	B	<1
β-Sitosterol	$C_{29}H_{50}O$	83-46-5	10.73	sterol	B	C

continued

α-tocopherol	$C_{29}H_{50}O_2$	59-02-9	10.96	tocopherol	B	C
γ-tocopherol	$C_{28}H_{48}O_2$	119-13-1	11.44	tocopherol	C	<1
squalene	$C_{30}H_{50}$	7683-64-9	12.19	terpenoid	B	<1
ferulic acid	$C_{10}H_{10}O_4$	1135-24-6	1.64	aromatic carboxylic	C	C
p-coumaric acid	$C_9H_8O_3$	7400-08-0	1.88	aromatic carboxylic	C	C
sucrose	$C_{12}H_{22}O_{11}$	57-50-1	-4.49	sugar	B	B
glucose	$C_6H_{12}O_6$	50-99-7	-2.49	sugar	C	C

[a] A: ≧1000 mg/kg, B: ≧10 mg/kg, < 1000 mg/kg, C: ≧1 mg/kg, < 10 mg/kg, <1: <1 mg/kg

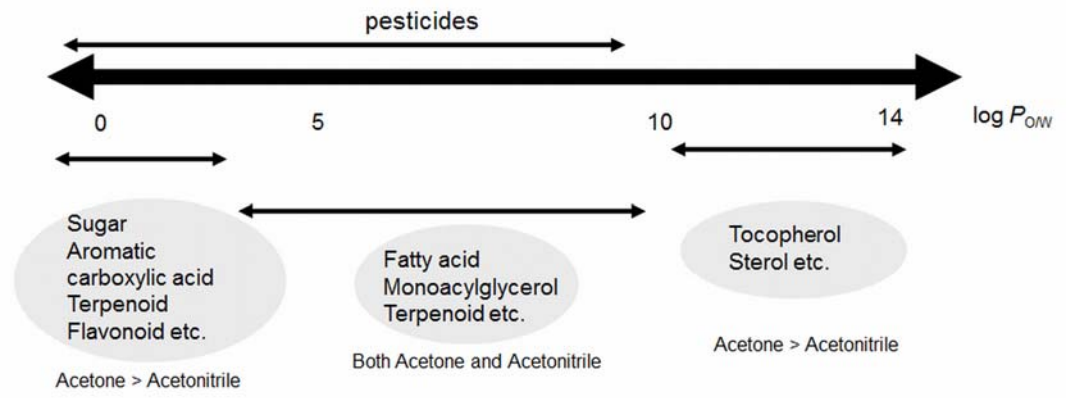


Fig. 4.4 Relationship between each component and log $P_{O/W}$

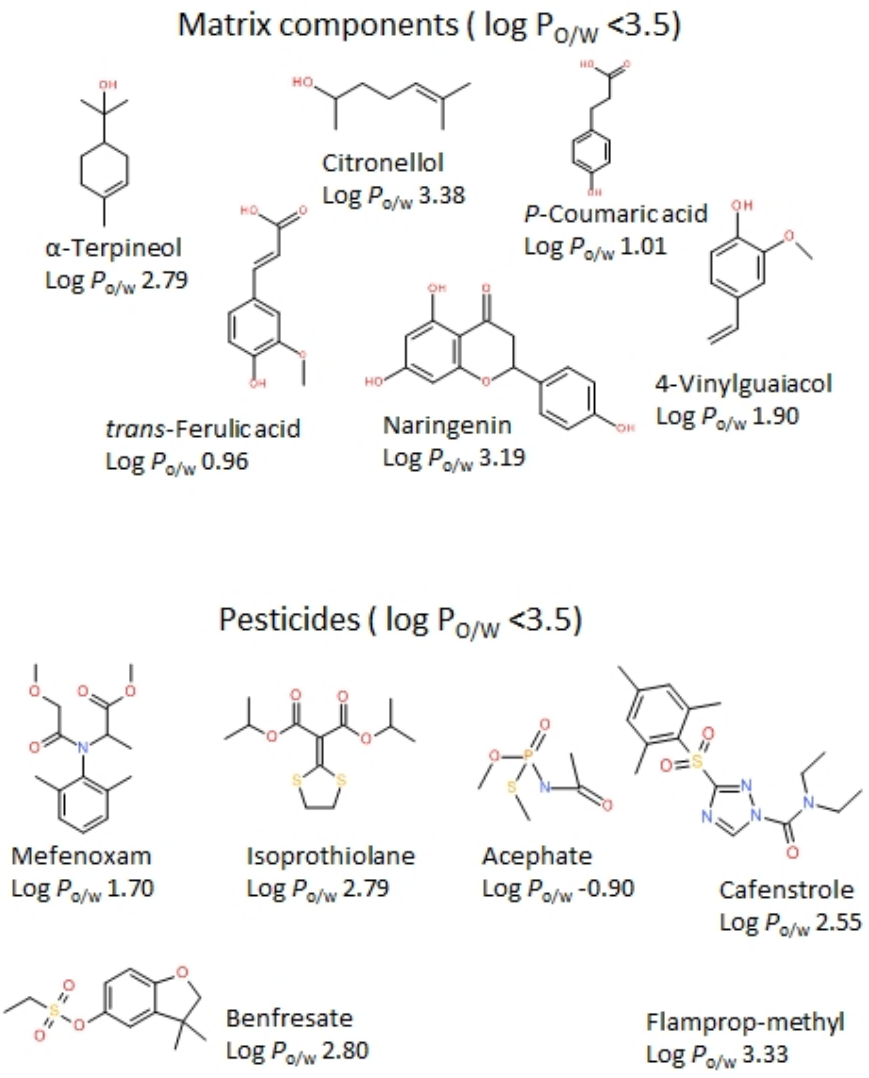


Fig. 4.5 Structures of matrix components and pesticides with log $P_{O/W}$ < 3.5.

components with log $P_{O/W}$ values > 10, such as sterols, were more extracted by acetone than by acetonitrile. The different extraction ability of low polar components influenced the next cleanup. The C18 column is not only used in place of the liquid-liquid extraction but also used for removing low polar compounds. Therefore, the combination of low extracting power of fat by acetonitrile and the removal ability of low polar components by the C18 column enabled the efficient removal of sterols from the samples. However, when acetone was used as the extraction solvent, excess sterols could not be completely removed by the C18 column. Compounds with log $P_{O/W}$ < 3.5, such as benzoic acid, 4-vinylguaiacol, *p*-coumaric acid, and some flavonoids, were more extracted by acetone than by acetonitrile. For these components, additional interactions, such as hydrogen bonds, might have worked, because both acetone and acetonitrile were high polar solvents. Fig. 4.5 shows the representative structure of matrix components and pesticides with log $P_{O/W}$ < 3.5. It was cleared that matrix components had hydroxyl or carboxyl group, otherwise pesticides not. Many kinds of terpenoids, which are characteristic compounds in orange, were extracted at the same concentration level by both acetone and acetonitrile, and their log $P_{O/W}$ values were in the range from 3.5 to 7. The log $P_{O/W}$ values of the monoacylglycerols, which are common compounds in all of the samples, are in the range from 5 to 7.5, and they were also extracted at the same concentration level by both acetone and acetonitrile. Glucose and fructose have two different structures, that is, a cyclic structure and a chain structure, and are in equilibrium in an aqueous solution. Most of the glucose and fructose exist as cyclic structures in an aqueous solution,[64)] but no data on their equilibrium states in an organic solvent were found. These cyclic sugars were extracted by both solvents at similar levels. However, the chain structure was found, and they were more extracted by acetonitrile than by acetone. In addition, dozens of unknown peaks of sugar-like

components were found in the orange sample. Although most sugar-like components are not listed in the database, they have the characteristic mass spectra of sugars; *m/z* 204, 217, 361, etc. These sugar-like components might be glycosides, which are bonded to other compounds or functional groups. Some of them were more extracted by acetone than by acetonitrile, whereas some of them were extracted by both solvents at the same level. The solvent, which easily dissolved these glycosides, might be dependent on the binding compounds.

On the basis of these results, acetone extracted the matrix components with a wide range of log $P_{O/W}$ values. In contrast, acetonitrile extracted the matrix components having log $P_{O/W}$ values were in the range from 3.2 to 10. The log $P_{O/W}$ values of the pesticides analyzed by GC-MS are mostly < 10. When acetonitrile was used as the extraction solvent, fewer low polar matrix components were extracted. Because both acetone and acetonitrile are high polar solvents, the polar matrix components should be dissolved in both solvents. However, some polar matrix components were more significantly extracted by acetone than by acetonitrile. There might be other interactions as described above. Therefore, acetone dissolved much of the polar matrix components. In fact, for many of the polar pesticides with log $P_{O/W}$ values < 3.2, good recovery rate of the multi-residue analysis using acetonitrile was proved by many previous studies.4-9

4.3.2 Experiment 2: Cleanup Efficiency by Solid Phase Extraction (SPE).

In experiment 2, the column cleanup efficiency was examined using several kinds of SPE columns. Because orange contained too many matrices, and it was biased to glycosides, the brown rice sample extracted using acetone was selected as the model sample in this experiment. The reason for using acetone extraction was that it was suitable to evaluate the column efficiency due to the many types of matrix components

from the result of experiment 1. All of the results are shown in Table 4.5.

Fatty acids, which are the main matrix components in the brown rice, have both a nonpolar hydrocarbon group and polar carboxyl group. Fatty acids were eluted from the silica gel column with *n*-hexane/acetone (15:85, v/v), while removed by a Florisil column because the polar interactions of Florisil are greater than that of the silica gel column. Both the NH_2 and PSA columns are used to exclude fats, but the PSA column showed a higher cleanup efficiency than the NH_2 column, and this result agreed with the report by Okihashi *et al.*[45] Fig. 4.6 shows the bar graph of elution rate from each column.

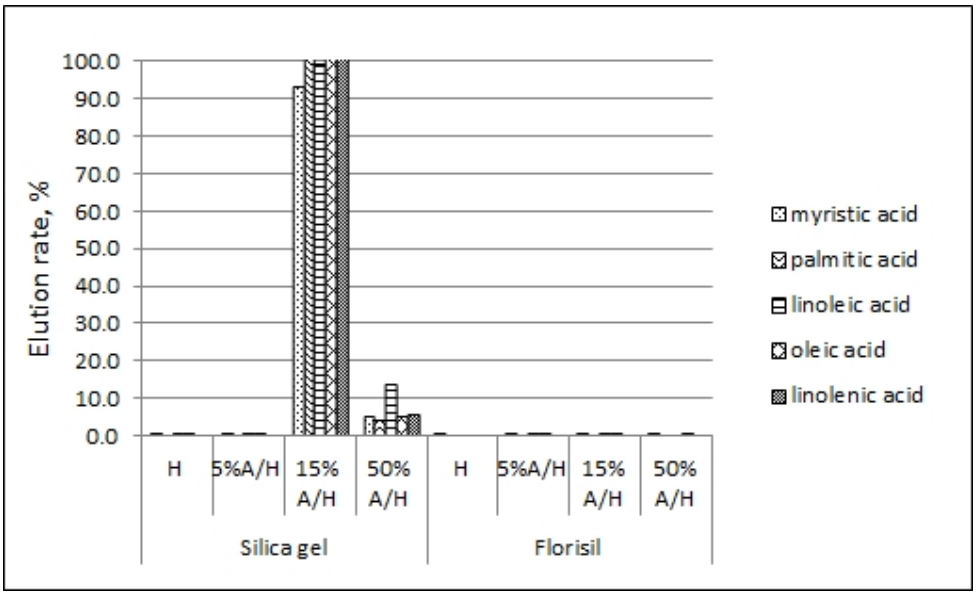
Sugars contain many hydroxyl groups (–OH) and water-soluble components. Sugars were removed by all columns except the GCB column. In addition, sugars do not move to the organic solvent layer when liquid-liquid extraction is applied during the general method of pesticide residue analysis.

Two carboxylic acids, p-coumaric acid and ferulic acid, were found in the brown rice extraction, and their structures were similar; both contain a phenolic hydroxyl group and a carboxyl group. However, the removal rate of p-coumaric acid was higher than that of ferulic acid by any column.

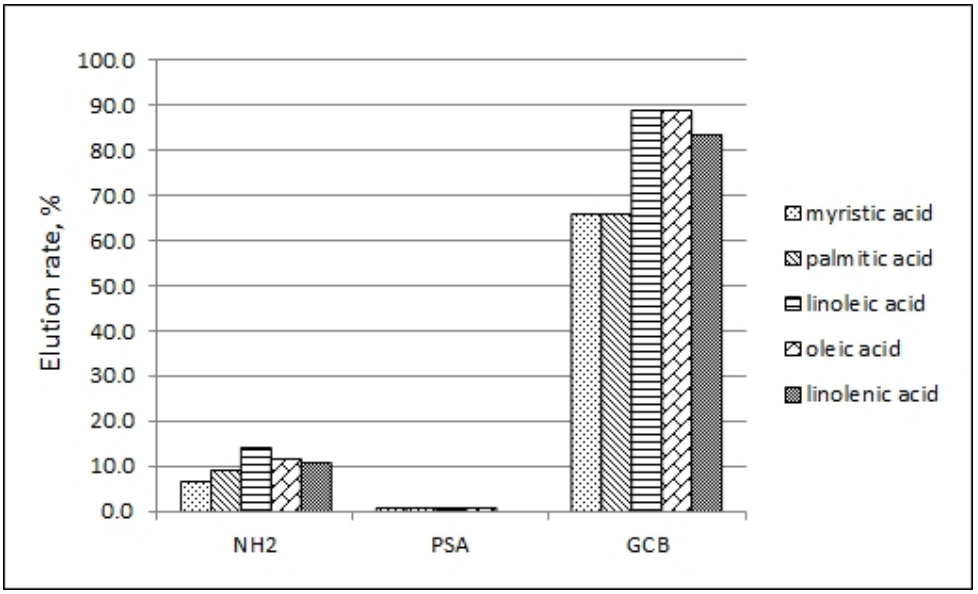
α-Tocopherol, γ-tocopherol and squalene are low polar compnents and were eluted from the silica gel and Florisil columns by acetone/*n*-hexane (5:95-15:85 v/v). The NH_2 and PSA columns did not effectively remove them. Only the GCB column removed them.

Monoacylglycerols are fat decomposition compounds and contain both a nonpolar hydrocarbon group and a polar hydroxyl group. Apporoximately 45-75% of the monoacylglycerols are eluted from any column. This result agreed with our other study.[31] We demonstrated that monoacylglycerols are the compounds that cause the

Individual (Group) analysis method



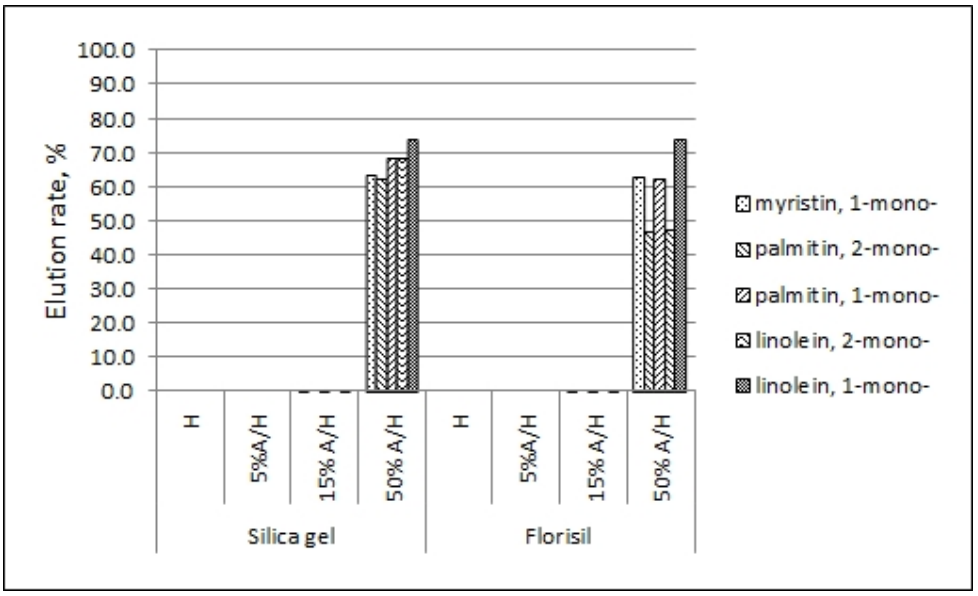
Multi-residue analysis method



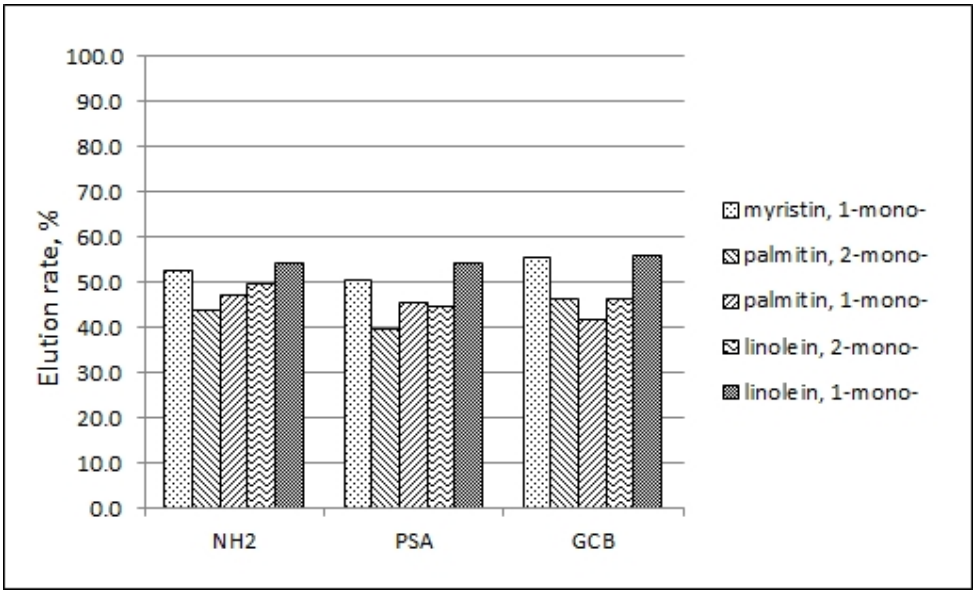
Elution rate (%) = (Mean value of the intensity of eluted matrix component from each column/mean value of the intensity of the components passed through the C18 column)×100

Fig. 4.6 Elution rate (%) of fatty acids from each column.

Individual (Group) analysis method



Multi-residue analysis method



Elution rate (%) = (Mean value of the intensity of eluted matrix component from each column/mean value of the intensity of the components passed through the C18 column)×100

Fig. 4.7 Elution rate (%) of monoacylglycerols from each column.

Table 4.5 Elution rate (%)[a] of Matrix Components from Each Column.

	Silica gel				Florisil				NH_2	PSA	GCB
	Fr-1	Fr-2 [c]	Fr-3 [d]	Fr-4 [e]	Fr-1	Fr-2 [c]	Fr-3 [d]	Fr-4 [e]			
Fatty acid											
lauric acid	0.0	0.0	103	7.9	0.0	0.3	0.4	0.3	5.6	0.4	61.6
myristic acid	0.1	0.1	93.0	5.4	0.1	0.1	0.1	0.1	6.3	0.1	65.8
pentadecanoic	0.4	1.0	93.7	5.4	0.3	0.8	0.6	0.9	6.2	0.6	55.2
palmitoleic acid	0.1	0.0	93.2	5.8	0.0	0.1	0.0	0.2	6.0	0.0	62.6
palmitic acid	0.1	0.3	113	4.4	0.1	0.4	0.3	0.2	8.9	0.2	65.5
heptadecanoic	0.0	0.0	97.7	3.0	0.0	0.0	0.0	0.0	2.6	0.0	50.8
heptadecenoic	0.0	1.8	93.7	4.4	0.3	1.9	2.0	1.1	8.6	0.0	50.2
linoleic acid	0.0	0.1	99.4	13.9	0.0	0.1	0.1	0.0	13.9	0.1	88.6
oleic acid	0.1	0.3	112	5.1	0.0	0.4	0.3	0.1	11.2	0.3	88.5
linolenic acid	0.0	0.0	111	5.3	0.0	0.0	0.0	0.0	10.4	0.0	83.0
stearic acid	0.5	1.3	94.8	3.4	0.4	1.3	1.4	0.9	11.2	0.9	39.0
11-eicosenoic acid	0.0	0.2	90.0	3.2	0.0	0.3	0.3	0.4	8.2	0.3	50.2
arachidic acid	0.5	1.1	95.5	3.5	0.3	1.2	1.3	1.2	9.7	1.5	1.3
behenic acid	0.0	0.5	86.0	2.6	0.1	0.4	0.5	0.0	10.5	0.0	1.4
lignoceric acid	0.0	0.3	97.8	2.0	0.0	0.7	0.6	0.8	14.9	1.4	0.8
Sugar											
glucose	0.0	0.0	0.0	11.6	0.0	0.0	0.0	0.0	0.0	0.0	76.3
sucrose	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.0	1.3	0.1	73.2
Monoacylglycero											
myristin, 1-mono-	0.0	0.0	0.2	63.1	0.0	0.0	0.1	62.7	52.7	50.6	55.7
palmitin, 2-mono-	0.0	0.0	0.0	62.1	0.0	0.0	0.0	46.8	43.9	39.9	46.4
palmitin, 1-mono-	0.0	0.0	0.2	68.0	0.0	0.0	0.2	62.3	47.1	45.6	41.7
linolein, 2-mono-	0.0	0.0	0.0	68.5	0.0	0.0	0.0	47.2	49.8	44.9	46.4
linolein, 1-mono-	0.0	0.0	0.2	73.9	0.0	0.0	0.1	74.0	54.2	54.3	55.8
Tocopherol											
α-tocopherol	0.0	97.0	6.6	0.1	0.0	85.2	2.5	0.1	63.2	80.2	0.1
γ-tocopherol	0.0	0.0	88.4	0.0	0.0	13.0	31.8	0.0	55.5	56.9	0.0

continued

Carboxylic acid											
p-coumaric acid	4.0	0.9	2.4	5.3	0.4	0.0	0.9	1.6	10.9	1.7	2.1
ferulic acid	5.2	4.8	72.2	46.1	5.0	5.9	7.6	85.6	99.1	51.7	12.7
Squalene											
squalene	15.1	32.0	1.9	0.2	1.7	81.6	0.9	0.3	72.9	69.1	8.3
Sterol											
campesterol	0.0	0.7	73.2	0.8	0.0	8.3	74.2	0.8	82.7	59.4	0.0
β-Sitosterol	0.0	0.4	76.0	0.2	0.0	7.7	75.3	0.3	84.0	59.8	0.0
stigmasterol	0.0	0.0	79.2	0.0	0.0	4.3	72.5	0.0	74.3	54.1	0.0
sterol-1 [f]	0.0	50.6	28.1	0.0	0.0	63.4	18.7	0.0	75.3	58.3	0.0
sterol-2 [g]	0.0	51.9	25.5	0.0	0.0	66.5	16.2	0.0	79.7	63.3	0.0

[a] (Mean value of the intensity of eluted matrix component from each column/Mean value of the intensity of the components passed through the C18 column) x 100

[b] Fr-1: *n*-hexane

[c] Fr-2: acetone/*n*-hexane (5:95, v/v)

[d] Fr-3: acetone/*n*-hexane (15:85, v/v)

[e] Fr-4: acetone/*n*-hexane (50:50, v/v)

[f] sterol-1: 9,19-Cyclolanost-24-en-3-ol, (3β)-

[g] sterol-2: 9,19-Cyclolanosta n-3-ol, 24-methylene-,(3β)-

matrix enhancement effect and remain at about 100 mg/kg in the sample solution when using the multi-residue method of the PLS, which adopts the combination of the GCB and NH_2column cleanup. Because monoacylglycerols are mid-polar components and their molecular weights are around 300-360, they might behave in the same manner as some pesticides. Although the Florisil and silica gel columns are rarely used for the multi-residue analysis because of their strong adsorption, there is the ability to adjust the proper ratio of the solvent mixture to remove the monoacylglycerols. In fact, Iijima *et al*. demonstrated the use of the silica gel column for multi-residue analysis, and most pesticides were eluted with acetone/*n*-hexane (15:85, v/v)[65]. Otherwise, searching for a suitable column to remove them is required.

Because sterols are low polar components, they were eluted from the Florisil and silica gel columns. The NH_2 and PSA columns could not sufficiently remove them, while the GCB column removed them because of their flat structures. In addition, a C18 column is used in the PLS method (used for grains, seeds, and beans) to remove fats.

4.3.3 Conclusions

Fig. 4.8 shows the matrix behavior in multi-residue analysis of pesticides that was cleared in this study. Although 90% of the fatty acids were removed by C18, fatty acids were still in the main matrices from the result of experiment 1. Main matrices were removed by either column except for the monoacylglycerols causing matrix enhancement effect. Next study is to remove monoacylglycerols. Examining the matrix components like this study can be helpful in designing the extraction and cleanup procedures (e.g., types of columns or elution solvent). This approach is also helpful for evaluating the method for other agricultural products (or biological samples), and to develop a better analytical method.

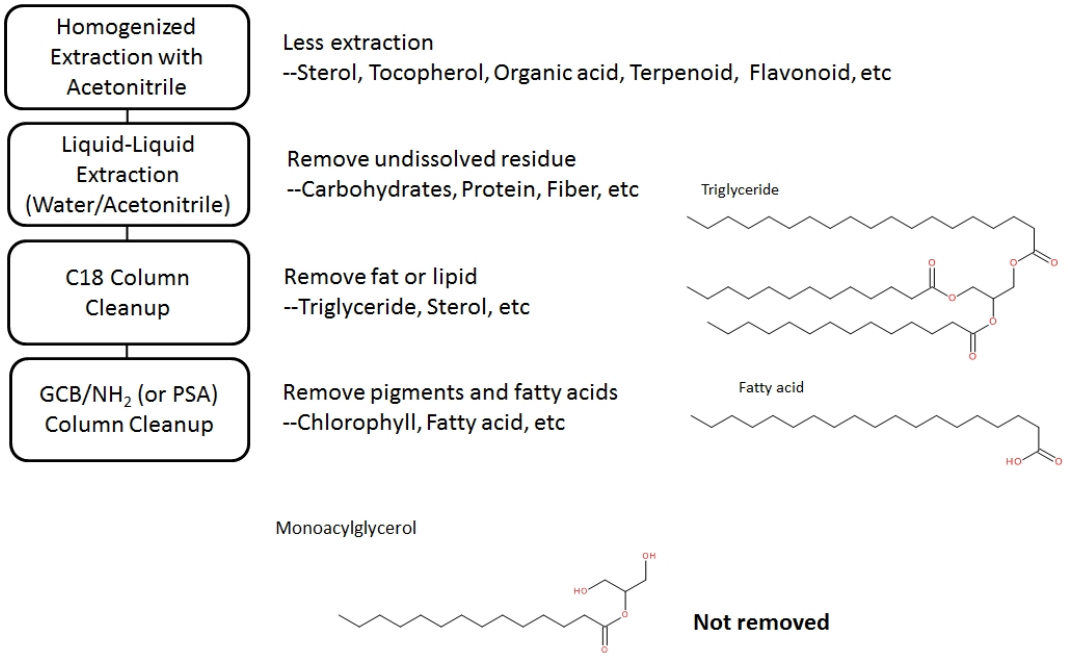


Fig. 4.8 Matrix behavior in multi-residue analysis of pesticides.

Summary

The objective of this thesis was to clarify the phenomenon that occurred in the GC-MS and influenced the determination of the trace levels of pesticides in the environmental and food safety fields. The following results were obtained.

Chapter 1 described the change in the mass spectra of fenthion sulfoxide, fenthion oxon sulfoxide and fensulfothion. These phenomena were caused by deoxidation in the ion source from the result of the product ion scan experiments. The author found that by adding PEG 300, it prevented the sulfoxide deoxidation. In addition, adding PEG 300 also compensated for the matrix enhancement effect including the other fenthion metabolites; i.e., fenthion oxon, fenthion sulfone and fenthion oxon sulfone.

Chapter 2 described the gold-plated ion source. The author made a gold-plated ion source and evaluated its performance. It is said that the matrix enhancement effect occurs in the injection port, column and ion source where the pesticides pass through. The author selected the most inert injection liner, the Siltek deactivated injection liner (Restek, Bellefonte, PA, USA) and the most inert column, factorFOUR VF-5ms (Agilent, Middleburg, Netherlands). The matrix enhancement of 60 representative pesticides in potato, spinach, orange, brown rice and soybean using the original ion source were 132, 202, 181, 240 and 151%, respectively. These values decreased to 129, 173, 145, 149 and 129%, respectively, using the gold-plated ion source. The amount of matrices in the measured solutions influenced the matrix enhancement effect and the effect was reduced for these samples. However, the matrix enhancement was not completely controlled by the gold-plated ion source.

Chapter 3 described the matrix components causing the matrix enhancement effect. From the result of Chapter 2, the author examined which matrix components cause the

matrix enhancement effect. No one has investigated which matrix components caused the matrix enhancement effect, because these types of matrix components seemed to be different depending on the samples. However, the author found that some common types of matrix components remained in the measured solutions, and the monoacylglycerols were the most attributable components.

Chapter 4 described the matrix behavior during sample preparation. As described in chapter 3, no one has investigated the matrix components for pesticide analysis. The author succeeded in applying the metabolic profiling analysis to determine the detailed matrix components. It became possible to know which matrix components were extracted and which matrix components were removed by the SPE column. In addition, the causative components of the matrix enhancement effect that were not removed was clarified. The author showed the importance of knowing the detailed matrix behavior in order to create a better analytical method.

In the future, the author plans to create a more inert ion source, not only gold-plating the ion source body and draw-out plate lens, but also the repeller and ion focus lens. From the aspect of the sample preparation, the author plans a unique cleanup. One is to synthesize acetonide from monoacylglycerols and acetone in an acetonitrile solution in order to reduce the polarity. If this reaction is successful, acetonide from monoacylglycerols may be removed by C18, the next step of the sample preparation for the pesticide residue analysis. Using diol columns and silica gel columns to remove the monoacylglcerols is another plan. Investigating the matrix components for other samples is also important.

References

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List of Publications

1) Sugitate, K., Yamagami, T., Nakamura, S., Toriba, A. and Hayakawa, K. Deoxidation of Fenthion Sulfoxide, Fenthion Oxon Sulfoxide and Fensulfothion in Gas Chromatograph/Mass Spectrometer, and the Prevention of Sulfoxide Deoxidation by Polyethylene Glycol 300. *Anal. Sci*. **2012**, *28*, 669-673.
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3) Sugitate, K., Nakamura, S., Orikata, N., Mizukoshi, K., Nakamura, M., Toriba, A. and Hayakawa, K. Search of components causing matrix effects on GC/MS for pesticide analysis in food. *J. Pestic. Sci*. **2012**, *37*, 156-163.
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